Vol. II No. 3

PLANT PHYSIOLOGY

JULY, 1927

CARBOHYDRATE TRANSFORMATIONS IN CARROTS DURING STORAGE

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Introduction

This paper gives the results of a study of changes taking place in carrots during storage. The changes investigated comprise the loss of water and solids and the variations in the relative proportions of sucrose, hexoses, and polysaccharides.

These changes were studied under two conditions of temperature; the one, 39° to 40° F., representing fairly well the average temperature of ordinary farm root-cellars, and the other, 32° to 35° F., approximating that usually employed in commercial cold storage for vegetables. As it was not possible, on account of the multiplicity of details, to conduct the experiments at both temperatures during one season, the experiments at the higher temperature were carried out during the season of 1924–25, and those at the lower temperature during 1925–26.

Nine varieties, representing the chief commercial types of stock and of table carrots, were studied under each temperature condition. With one exception, the same varieties were used in both cases.

The work was made possible through the cooperation of Dr. J. I. LAURITZEN, whose investigations on the effects of different storage conditions on the growth of organisms causing decay of carrots afforded an opportunity of obtaining material grown especially for the work and stored under controlled conditions.

Historical

The numerous analyses of carrots giving the proximate composition according to the Weende routine need not be especially mentioned here.

They are mostly to be found in the compilations of KÖNIG (**11**), JENKINS (**9**), JENKINS and WINTON (**10**), ATWATER (**1**), ATWATER and WOODS (**2**), and ATWATER and BRYANT (**3**), and in the reports of WERENSKIOLD (**21**) and of SHUTT (**20**). It may be of interest, however, as showing the range and variability of reducing sugars and sucrose in carrots to cite a few analyses in which these have been separately determined. As early as 1860 DIETRICH (**7**) gave for three varieties of carrots a dextrose content of 2.95, 8.09, and 4.84 per cent., and a corresponding sucrose content of 6.60, 3.99, and 4.16 per cent. RITTHAUSEN (**17**) reports analyses by FUNK showing 0.86 per cent. of crude sugar and 2.76 per cent. of dextrose in the green-shouldered white stock carrot, and 1.59 per cent. of crude sugar and 3.92 per cent. of dextrose in a red variety. According to the data given by WERENSKIOLD (**21**) for several varieties grown in Norway in 1894 and 1895, the reducing-sugar content ranged from 1.58 to 4.25 per cent., and the sucrose content from 1.98 to 4.94 per cent. KRISTOFFERSON (**12**) also found that the sucrose content and the invert-sugar content vary greatly in different varieties as well as in individual carrots of the same variety. The invert-sugar content of six varieties and strains ranged from 2.21 to 4.53 per cent., while the sucrose content recalculated from his figures varied from 2.59 to 5.81 per cent. MYERS and CROLL (**14**) found 6.20 per cent. of reducing-sugar and 1.30 per cent. of sucrose. It is thus apparent that there is considerable variability in the sugar content of carrots, even within the same variety.

The analyses so far reported in the literature have all been made merely from the standpoint of determining the constituents of the roots, usually without reference to previous treatment of the material. So far as I have been able to determine, no systematic analyses for the purpose of showing changes in the constituents during storage have been made.

Of the carbohydrates of the carrot, sucrose has been identified by SCHMIDT (**19**) and dextrose by BUSOLT (**6**). The occurrence of starch, as a rule, is not mentioned. Occasionally its absence is noted (**19**), yet NESSLER (**15**), according to KÖNIG, reports 0.22 per cent. of starch and BAESSLER (**4**) gives 0.92 per cent. for small roots and 0.87 per cent. for large ones. Details as to identification are lacking. FALK (**8**), also without specific identification, reports as insoluble starch the difference between the total carbohydrates after hydrolysis and the soluble carbohydrates all in terms of cuprous oxide. In the varieties used in the present investigation I have not been able to show the occurrence of granular starch. It seems, therefore, that the reducing sugars obtained by hydrolysis after extraction with 90 per cent. alcohol are derived from dextrins and hemicelluloses.

Experimental procedure

The carrots used in the present investigation were all grown in the same field on sandy loam at the Arlington experimental farm in Virginia during the summers of 1924 and 1925.

At the time of harvesting two varieties were dug each day until the harvesting was completed. The roots were washed immediately after they had been dug and were then allowed to dry over night in a cool room. On the following morning four lots of about five to six kilograms each were weighed out for each variety and put into baskets, over which heavy manila covers were then tied. One basket of each variety was immediately taken to the laboratory and prepared for analysis. The other three baskets were put into storage at the desired temperature in a room at the experimental cold storage plant at the Arlington farm. At intervals the lots of stored carrots were weighed. One basket of each variety was taken to the laboratory for analysis. The rest were carefully examined. Carrots which showed decayed spots or other signs of deterioration indicating that they might not remain in good condition over the next storage period were discarded after a record of the extent of the injuries had been made. The sound carrots remaining were reweighed and replaced in the baskets. The new weight was taken as a basis for determining the loss of weight during the next storage period. This procedure was continued until the last basket of each variety had been used for analysis.

The record of these weighings, which were made by Dr. LAURITZEN, is given in tables I and II, in order to show the actual quantities upon which the subsequent calculations and determinations were based.

The record of the extent of infection and sprouting among the stored carrots served as a basis for judging the degree to which these factors might have influenced the loss of water and solid matter from the roots. It is safe to say that this influence was practically negligible. Infected carrots occurred only sporadically, mostly during the early part of the storage season. Some varieties had no infection. As a rule, only one or two infected carrots were found in any lot, rarely more. The infections were usually limited to spots which varied from 2 to 15 millimeters in length, and only in isolated cases, when the root-tip was involved, exceeded this length. Sprouting occurred during the last storage period in a few roots of some of the varieties at the higher temperature (39° to 40° F.). The young leaves ranged from 2 to 7 centimeters in length. A comparison of the water loss from lots containing sprouted carrots with that from lots with no sprouted carrots showed no appreciable effect of the slight development of sprouts on the loss of water. The sprouted carrots were not used for analysis.

The empirical data in tables I and II do not show at a glance the comparative losses of material from the different lots of stored carrots. There-

TABLE I

WEIGHTS OF DIFFERENT LOTS OF CARROTS AT SUCCESSIVE STAGES OF STORAGE AT 39° TO 40° F.

VARIETY	AT BEGINNING OF STORAGE		AFTER 67–69 DAYS' STORAGE				AFTER 102–105 DAYS' STORAGE				AFTER 153–155 DAYS' STORAGE		
	DATE 1924	WEIGHT WHEN PLACED IN STORAGE	DATE 1925	TOTAL WEIGHT OF CARROTS	WEIGHT MINUS DISCARDED CARROTS*	LOSS OF WATER AND SOLIDS	DATE 1925	TOTAL WEIGHT OF CARROTS	WEIGHT MINUS DISCARDED CARROTS*	LOSS OF WATER AND SOLIDS	DATE 1925	TOTAL WEIGHT OF CARROTS	LOSS OF WATER AND SOLIDS
Danvers Half Long	Oct.	gm.	Jan.	gm.	gm.	gm.	Feb.	gm.	gm.	gm.	April	gm.	gm.
"	28	6456	5	5800	....	656							
"		6478		5812	....	666	9	5810†	....	2			
Blanche lisse demi-longue		6458		5760	....	698		5473	....	287	1	4887	586
"	28	6447	5	5813	....	634							
"		6437		5775	4020	662	9	3815	....	205			
Jaune obtuse du Doubs		6433		5793	3539	640		3341	....	198	1	2916	425
"	29	6495	6	5915	....	580							
"		6485		5700	5396	785	10	5132	....	264			
Carter's Scarlet Perfection		4608		3870	3559	738		3335	....	224	2	2911	424
"	29	6490	6	5816	....	674							
"		6429		5800	5700	629	10	5404	....	296			
Rouge demi-longue de Chatenay		2889		2505	....	384		2348	2296	157	2	1922	374
"	30	6424	7	5759	....	665							
"		6493		5939	5187	554	11	4905	....	282			
Carter's Summer Favorite		6479		5692	5627	787		5362	....	265	3	4820	542
"	30	6460	7	5770	....	690							
"		6482		5901	....	581	11	5636	....	265			
Carter's Early Market		6500		5940	....	560		5670	....	270	3	5088	582
"	Nov. 1	6433	9	5911	....	522							
"		6482		5967	....	515	13	5647	....	320			
Carter's Red Elephant		6488		5892	....	596		5596	....	296	4	5007	589
"	1	6458	9	5855	....	603							
"		5525		5027	4935	498	14	4636	....	299			
Blanche à collet vert		5588		4967	....	621		4657	....	310	4	4098	559
"	4	6470	10	5819	....	651							
"		6447		5719	....	728	14	5428	....	291			
"		6490		6031	5673	459		5370	....	303	6	4804	566

* Where figures appear in this column carrots were discarded, on account of infection, from the baskets continued in storage.
† This figure was not used in the calculation of the corresponding average in table III.

† This figure was not used in the calculation of the corresponding average in table III.

TABLE II

WEIGHTS OF DIFFERENT LOTS OF CARROTS AT SUCCESSIVE STAGES OF STORAGE AT 32° TO 35° F.

VARIETY	AT BEGINNING OF STORAGE		AFTER 65–68 DAYS' STORAGE				AFTER 100–103 DAYS' STORAGE				AFTER 150–155 DAYS' STORAGE		
	DATE 1925	WEIGHT WHEN PLACED IN STORAGE	DATE 1926	TOTAL WEIGHT OF CARROTS	WEIGHT MINUS DISCARDED CARROTS*	LOSS OF WATER AND SOLIDS	DATE 1926	TOTAL WEIGHT OF CARROTS	WEIGHT MINUS DISCARDED CARROTS*	LOSS OF WATER AND SOLIDS	DATE 1926	TOTAL WEIGHT OF CARROTS	LOSS OF WATER AND SOLIDS
	Nov.	gm.	Jan.	gm.	gm.	gm.	Feb.	gm.	gm.	gm.	April	gm.	gm.
Danvers Half Long	4	6490	11	6255	....	235							
"		6469		6148	....	321	15	6055	....	93			
"		6500		6266	....	234		6168	5929	98	6	5874	55
Blanche lisse demi-longue	4	6474	11	6192	....	282							
"		6490		6151	5849	339	15	5721	....	128			
"		6471		6219	....	252		6100	5985	119	6	5857	128
Jaune obtuse du Doubs	5	6491	12	6134	....	357							
"		6438		6187	6130	251	16	6004	....	126			
"		6458		6235	6143	223		6000	....	143	7	5935	65
Carter's Scarlet Perfection	5	6457	12	6192	....	265							
"		6477		6140	....	337	16	6039	....	101			
"		6429		6092	....	337		5977	....	115	7	5853	124
Carter's Summer Favorite	6	6418	13	6164	....	254							
"		6436		6194	5881	242	17	5765	....	116			
"		6469		6137	5930	332		5827	....	103	8	5710	117
Carter's Red Elephant	7	6450	13	6112	....	338							
"		6417		6167	....	250	17	6051	....	116			
"		6481		6257	6002	224		5980	....	22	8	5899	81
Carter's Early Market	7	6493	14	6206	....	287							
"		6470		6255	6166	215	18	6056	....	110			
"		6450		6125	....	325		6005	....	120	9	5901	104
Carter's Nantes	10	6450	14	6177	....	273							
"		6493		6185	....	308	18	6095	....	90			
"		6448		6227	....	221		6110	5767	117	9	5709	58
Blanche à collet vert	10	6447	15	6230	....	217							
"		6483		6190	6057	293	18	5935	....	122			
"		6481		6290	6111	191		5998	5654	113	10	5559	95

* Where figures appear in this column carrots were discarded, on account of infection, from the baskets continued in storage.

TABLE III

SHRINKAGE, ON BASIS OF 100 AS ORIGINAL WEIGHT, OF CARROTS STORED AT 39° TO 40° F.

VARIETY	WEIGHT (EQUIVALENT TO 100), WATER CONTENT AND SOLIDS AT BEGINNING OF STORAGE				WEIGHT, WATER CONTENT AND SOLIDS AFTER 67–69 DAYS' STORAGE					WEIGHT, WATER CONTENT AND SOLIDS AFTER 102–105 DAYS' STORAGE					WEIGHT, WATER CONTENT AND SOLIDS AFTER 153–155 DAYS' STORAGE				
	DATE	ORIGINAL WEIGHT	WATER / SOLIDS	WATER / SOLIDS "A"	DATE	100 SHRUNK TO	WATER / SOLIDS	WATER / SOLIDS "B"	DECREASE IN WATER AND SOLIDS IN CARROTS "A" MINUS "B"	DATE	100 SHRUNK TO	WATER / SOLIDS	WATER / SOLIDS "C"	DECREASE IN WATER AND SOLIDS IN CARROTS "B" MINUS "C"	DATE	100 SHRUNK TO	WATER / SOLIDS	WATER / SOLIDS "D"	DECREASE IN WATER AND SOLIDS IN CARROTS "C" MINUS "D"
	Oct.		Per cent.	Actual	Jan.		Per cent.	Actual	Actual	Feb.		Per cent.	Actual	Actual	Apr.		Per cent.	Actual	Actual
Danvers Half Long	28	100	88.07 / 11.93	88.07 / 11.93	5	89.58	87.62 / 12.38	78.49 / 11.09	9.58 / 0.84	9	84.75	87.03 / 12.97	73.76 / 10.99	4.73 / 0.10	1	75.67	85.50 / 14.50	64.70 / 10.97	9.06 / 0.02
Blanche lisse demi-longue..	28	100	90.12 / 9.88	90.12 / 9.88	5	89.98	89.46 / 10.54	80.50 / 9.48	9.62 / 0.40	9	85.08	88.78 / 11.22	75.53 / 9.55	4.97 / +0.07	1	74.21	88.41 / 11.59	65.61 / 8.60	9.92 / 0.95
Jaune obtuse du Doubs ...	29	100	88.94 / 11.06	88.94 / 11.06	6	87.65	88.45 / 11.55	77.53 / 10.12	11.41 / 0.94	10	81.15	87.44 / 12.56	70.96 / 10.19	6.57 / +0.07	2	68.70	86.69 / 13.31	59.56 / 9.14	11.40 / 1.05
Carter's Scarlet Perfection ..	29	100	87.90 / 12.10	87.90 / 12.10	6	88.85	87.44 / 12.56	77.69 / 11.16	10.21 / 0.94	10	83.41	86.88 / 13.12	72.47 / 10.94	5.22 / 0.22	2	68.03	83.74 / 16.26	56.97 / 11.06	15.50 / +0.12
Rouge demi-longue de Chantenay...	30	100	88.87 / 11.13	88.87 / 11.13	7	89.66	88.59 / 11.41	79.43 / 10.23	9.44 / 0.90	11	85.10	88.55 / 11.45	75.36 / 9.74	4.07 / 0.49	3	75.25	87.13 / 12.87	65.57 / 9.68	9.79 / 0.06
Carter's Summer Favorite	30	100	89.35 / 10.65	89.35 / 10.65	7	90.58	88.61 / 11.39	80.26 / 10.32	9.09 / 0.33	11	87.09	88.52 / 11.48	77.09 / 10.00	3.17 / 0.32	3	78.27	87.81 / 12.19	68.73 / 9.54	8.36 / 0.46
	Nov.																		
Carter's Early Market	1	100	88.92 / 11.08	88.92 / 11.08	9	91.58	88.42 / 11.58	80.98 / 10.60	7.94 / 0.48	13	86.69	88.12 / 11.88	76.39 / 10.30	4.59 / 0.30	4	77.17	86.69 / 13.31	66.90 / 10.27	9.49 / 0.03
Carter's Red Elephant ...	1	100	87.23 / 12.77	87.23 / 12.77	9	90.18	86.72 / 13.28	78.20 / 11.98	9.03 / 0.79	14	84.41	86.01 / 13.99	72.60 / 11.81	5.60 / 0.17	4	73.34	84.77 / 15.23	62.17 / 11.17	10.43 / 0.64
Blanche à collet vert ..	4	100	89.98 / 10.02	89.98 / 10.02	10	90.53	89.06 / 10.94	80.63 / 9.90	9.35 / 0.12	14	86.08	88.53 / 11.47	76.21 / 9.87	4.42 / 0.03	6	78.70	87.87 / 12.13	69.15 / 9.55	7.06 / 0.32

fore, the figures have all been reduced to the basis of an original weight of 100 in tables III and IV. These figures show directly the shrinkage during each storage period and also permit of an immediate comparison of the shrinkage under the two storage conditions.

Other data in these tables show the percentage of water and of solids in the carrots at the different stages of storage, the actual quantities of these substances on the basis of 100 taken as the original weight of the roots, and the decrease during each storage interval in the water and solids. These data are based on averages of the figures in tables I and II, wherever more than one determination was made.

The data in tables III and IV show that at the lower storage temperature the carrots lost on the average about 7 per cent. of their weight during the entire storage season, and at the higher temperature about 26 per cent. These losses consisted mostly of water. The loss of solid matter at the lower temperature amounted to about 9.5 per cent. of the original solids, and at the higher temperature to 10.5 per cent., or about one per cent. of the total weight of the carrots. The greater part of this loss occurred during the first two months of storage. The figures relating to the loss of solids are probably not entirely accurate in detail. The positive increments (marked by a + sign in the tables) show that in a few cases the error of sampling exceeded in magnitude the loss of solid material. Nevertheless, the general agreement of the figures in each column indicates that they represent fairly well the loss through respiration.

The figures giving the differences between the water present in the roots at the beginning and at the end of each storage period show approximately the water loss during each period. To give the true loss they should be increased by the amounts representing the water formed through respiration. On the assumption that the respiratory material is glucose and that it is completely oxidized, this increase would be equal to 60 per cent. of the solid matter lost.[1]

Analytical methods

(*a*) *Preparation of the material.*—In the preparation of the carrots for analysis, the whole of the lot except the infected and the sprouted carrots, which were discarded, was used as a sample in every case. The crowns were cut off to remove the leaf-bases. Large carrots were split once or twice, and all were cut into short pieces with a rotary slicer. The pieces were then rapidly mixed and ground through a power-driven meat grinder having a face-plate with holes 3.2 mm. in diameter. The vessel containing

[1] Theoretically, a slight further correction should be made both in the figures for the loss of solids and in the approximate figures representing the loss of water, on account of water bound by hydrolytic processes. Both errors are insignificant in the present work.

TABLE IV

SHRINKAGE, ON BASIS OF 100 AS ORIGINAL WEIGHT, OF CARROTS STORED AT 32° TO 35° F.

VARIETY	WEIGHT (EQUIVALENT TO 100), WATER CONTENT AND SOLIDS AT BEGINNING OF STORAGE				WEIGHT, WATER CONTENT AND SOLIDS AFTER 65–68 DAYS' STORAGE					WEIGHT, WATER CONTENT AND SOLIDS AFTER 100–103 DAYS' STORAGE					WEIGHT, WATER CONTENT AND SOLIDS AFTER 150–155 DAYS' STORAGE				
	DATE	ORIGINAL WEIGHT	WATER / SOLIDS	WATER / SOLIDS "A"	DATE	100 SHRUNK TO	WATER / SOLIDS	WATER / SOLIDS "B"	DECREASE IN WATER AND SOLIDS IN CARROTS "A" MINUS "B"	DATE	100 SHRUNK TO	WATER / SOLIDS	WATER / SOLIDS "C"	DECREASE IN WATER AND SOLIDS IN CARROTS "B" MINUS "C"	DATE	100 SHRUNK TO	WATER / SOLIDS	WATER / SOLIDS "D"	DECREASE IN WATER AND SOLIDS IN CARROTS "C" MINUS "D"
	Nov.		Per cent.	Actual	Jan.		Per cent.	Actual	Actual	Feb.		Per cent.	Actual	Actual	Apr.		Per cent.	Actual	Actual
Danvers Half Long	4	100	88.74 / 11.26	88.74 / 11.26	11	95.94	88.86 / 11.14	85.25 / 10.69	3.49 / 0.57	15	94.25	89.10 / 10.90	83.98 / 10.27	1.27 / 0.42	6	94.02	89.25 / 10.75	83.91 / 10.11	0.07 / 0.16
Blanche lisse demi-longue..	4	100	89.68 / 10.32	89.68 / 10.32	11	95.51	90.29 / 9.71	86.24 / 9.27	3.44 / 1.05	15	93.49	90.07 / 9.93	84.21 / 9.28	2.03 / + 0.01	6	92.25	89.97 / 10.03	83.00 / 9.25	1.21 / 0.03
Jaune obtuse du Doubs ...	5	100	88.57 / 11.43	88.57 / 11.43	12	95.72	88.71 / 11.29	84.91 / 10.81	3.66 / 0.62	16	94.21	88.71 / 11.29	83.57 / 10.64	1.34 / 0.17	7	93.28	88.98 / 11.02	83.00 / 10.28	0.57 / 0.36
Carter's Scarlet Perfection ..	5	100	87.43 / 12.57	87.43 / 12.57	12	95.15	87.77 / 12.23	83.51 / 11.64	3.92 / 0.93	16	93.11	87.66 / 12.34	81.62 / 11.49	1.89 / 0.15	7	91.05	87.80 / 12.20	79.94 / 11.11	1.68 / 0.38
Carter's Summer Favorite	6	100	89.26 / 10.74	89.26 / 10.74	13	95.72	89.65 / 10.35	85.81 / 9.91	3.45 / 0.83	17	93.78	89.65 / 10.35	84.07 / 9.71	1.74 / 0.20	8	91.35	89.35 / 10.65	81.62 / 9.73	2.45 / + 0.02
Carter's Red Elephant ...	7	100	87.71 / 12.29	87.71 / 12.29	13	95.80	87.74 / 12.26	84.05 / 11.75	3.66 / 0.54	17	95.24	87.90 / 12.10	83.72 / 11.52	0.33 / 0.23	8	94.88	88.24 / 11.76	83.72 / 11.16	0.00 / 0.36
Carter's Early Market	7	100	88.19 / 11.81	88.19 / 11.81	14	95.74	88.19 / 11.81	84.43 / 11.31	3.76 / 0.50	18	94.03	88.27 / 11.73	83.00 / 11.03	1.43 / 0.28	9	91.49	88.33 / 11.67	80.81 / 10.68	2.19 / 0.35
Carter's Nantes	10	100	88.87 / 11.13	88.87 / 11.13	14	95.87	88.60 / 11.40	84.94 / 10.93	3.93 / 0.20	18	94.32	88.73 / 11.27	83.69 / 10.63	1.25 / 0.30	9	93.79	88.93 / 11.07	83.41 / 10.38	0.28 / 0.25
Blanche à collet vert ..	10	100	88.97 / 11.03	88.97 / 11.03	15	96.39	89.30 / 10.70	86.08 / 10.31	2.89 / 0.72	18	94.41	89.20 / 10.80	84.21 / 10.20	1.87 / 0.11	10	93.65	89.21 / 10.79	83.55 / 10.10	0.66 / 0.10

the cut pieces and the vessel receiving the pulp were kept covered with damp cloths. The pulp was rapidly mixed on a stone slab and quartered down to a workable quantity. From this were weighed out two 25-gram samples for sugar determinations, two 10-gram (25-gram in the second year's work) samples for the determination of polysaccharides, and two approximately 10-gram samples for moisture determinations. All samples were covered with neutral 95 per cent. alcohol as soon as they had been weighed out. To the beakers into which the sugar samples were weighed 0.25 of a gram of calcium carbonate had been previously added.

(*b*) *Determination of sugar.*—When the weighing had been completed, the sugar samples, to which enough alcohol had been added to bring the concentration to about 70 per cent., were washed into 250-ml. volumetric flasks with enough 70 per cent. alcohol to occupy about three-fourths of the volume of the flasks. The flasks were then placed in a hot water-bath and boiled for 15 minutes. Evaporation was reduced by means of empty calcium-chloride tubes loosely placed in the necks of the flasks.

The flasks containing the sugar samples were cooled, filled to the mark at 20° C. and set aside for several weeks until the determinations were begun. During that time they were frequently shaken. Since a little alcohol evaporated from the glass-stoppered flasks, they were cooled to 20° C. at intervals and refilled to the mark. After the last filling they were always allowed to stand a few days to ensure uniformity of concentration in the liquid and pulp. The extracts were subsequently treated essentially as in the alcohol extraction method described by BRYAN, GIVEN and STRAUGHN (**5**), 100-ml. portions being used for the operations.[2]

In the preparation of the alkaline tartrate solution and the copper sulphate solution, and in the reductions, the details of procedure as described by MUNSON and WALKER (**13**) were followed.

Beakers of approximately the same heat transmissivity were selected, according to the procedure of PETERS (**16**). To prevent the heating of the sides of the beakers in which the reductions were carried out, a ring of thick asbestos, cut to fit the beakers, was fastened to the asbestos gauze upon which they were heated. Before each set of determinations the flame was adjusted by trials, so as to bring the Fehling's mixture with the added sugar solution to boiling in the prescribed time. The cuprous oxide was weighed as such.

The volume occupied by the pulp, plus the undissolved calcium carbonate in the 250-ml. flasks, was determined by means of a pycnometer. Seventy per cent. alcohol of the same strength as that with which the residues had

[2] One ml. of a saturated solution of lead acetate [$Pb(C_2H_3O_2)_2 \cdot 3H_2O$] was found to be suitable for clearing 100 ml. portions of these filtrates, after the necessary preliminary steps. The excess of lead was finally removed with 0.2 of a gram of dry sodium oxalate.

been previously exhaustively extracted and washed was used as a medium. The results are given in table V.

TABLE V

VOLUME OCCUPIED BY CARROT PULP

VARIETY	VOLUME OCCUPIED BY INSOLUBLE RESIDUE IN 25 GM. FRESH PULP*	VOLUME OCCUPIED BY 1 GM. OF WATER-FREE EXTRACTED RESIDUE*	VOLUME OCCUPIED BY INSOLUBLE RESIDUE IN 25 GM. FRESH PULP*	VOLUME OCCUPIED BY 1 GM. OF WATER-FREE EXTRACTED RESIDUE*
	AT BEGINNING OF STORAGE		AFTER 153–155 DAYS' STORAGE	
	ml.	ml.	ml.	ml.
Danvers Half Long	0.51	0.40	0.60	0.44
Blanche lisse demi-longue	0.57	0.53	0.55	0.46
Jaune obtuse du Doubs	0.59	0.46	0.65	0.47
Carter's Scarlet Perfection	0.62	0.44	0.82	0.50
Rouge demi-longue de Chantenay	0.53	0.44	0.55	0.44
Carter's Summer Favorite	0.46	0.40	0.49	0.41
Carter's Early Market	0.51	0.45	0.60	0.46
Carter's Red Elephant	0.70	0.48	0.84	0.50
Blanche à collet vert	0.40	0.36	0.60	0.48
Average	0.54	0.44	0.63	0.46

* Plus residual calcium carbonate.

The average volume occupied by the pulp was about 0.6 ml. A correction for this would give for the maximum percentage of reducing sugar 4.13 instead of 4.14, and for the maximum percentage of total sugar calculated as dextrose 7.24 instead of 7.26. Since the second decimal in these percentages is not significant, it was not deemed necessary to apply the correction.

(*c*) *Determination of acid-hydrolysable polysaccharides.*—The samples for the determination of polysaccharides insoluble in 90 per cent. alcohol and hydrolysable by 0.695 N. hydrochloric acid were stored without boiling and without calcium carbonate in flasks with 200 or 350 ml. of 95 per cent. alcohol, according as 10-gram or 25-gram samples were used. They were extracted in Soxhlet apparatus with 90 per cent. alcohol. The residues were washed into Erlenmeyer flasks with 95 per cent. alcohol which was evaporated almost to dryness in the water bath. The hydrolyses were carried out essentially according to the method of SACHSSE (**18**), except that for the 10-gram samples 100 ml. of acid (0.695 N.), and for the 25-gram samples 200 ml., were added to the pulp. The flasks were heated under reflux condensers for three hours in a vigorously boiling water-bath. The resulting extracts were filtered from the residues, which were washed with many small portions of hot water until, as determined by preliminary tests on collateral samples, all sugar had been removed. The filtrates, collected directly in volumetric flasks of 250-ml. or 500-ml. capacity, according as 10-gram samples or 25-gram samples had been used, were treated with one ml. of phosphotungstic acid solution (10 per cent. phosphotungstic acid in one per cent. hydrochloric acid) and, after having been cooled to 20° C., were made up to the mark. Of the solution filtered from the phosphotungstic acid precipitate, 100 ml. were nearly neutralized by the addition of 4 ml. of sodium hydroxide solution, the neutralization being finished by the addition of a trace of anhydrous sodium carbonate. After filtration, the reducing sugars were determined. The increase in volume from 100 ml. to 104 ml. was taken into consideration in the calculations of the results.

The addition of one ml. of phosphotungstic acid to 250 ml. of the solution does not have any appreciable effect on the reduction. Blanks with neutralized acid solution, to which the same quantity of phosphotungstic acid had been added, gave 0.4, 0.0, and 0.4 mg. cuprous oxide, while blanks without phosphotungstic acid gave 0.2, 0.1, and 0.2 mg. of cuprous oxide.

(*d*) *Determination of moisture.*—For the determination of moisture the free alcohol was evaporated from the samples at 40° to 50° C. The residues were then dried to constant weight in a slow current of air at 80° C. and at a pressure of about 8 cm. of mercury.

Analytical results

The data obtained from the analyses are given in tables VI and VII. These tables give the carbohydrate content of the roots at different stages of storage under the two temperature conditions.

The changes which take place in the carbohydrates of the carrots are, of course, not truly represented by differences in percentage composition at different stages of storage, since a direct comparison of the percentages does

TABLE VI

COMPOSITION OF CARROTS STORED AT 39° TO 40° F.

VARIETY	AT BEGINNING OF STORAGE			AFTER 67–69 DAYS' STORAGE			AFTER 102–105 DAYS' STORAGE			AFTER 153–155 DAYS' STORAGE		
	REDUCING SUGAR	SUCROSE	ACID HYDROLYSABLE POLYSACCHARIDES	REDUCING SUGAR	SUCROSE	ACID HYDROLYSABLE POLYSACCHARIDES	REDUCING SUGAR	SUCROSE	ACID HYDROLYSABLE POLYSACCHARIDES	REDUCING SUGAR	SUCROSE	ACID HYDROLYSABLE POLYSACCHARIDES
	Per cent.	Per cent.	Per cent.	Per cent.	Per cent.	Per cent.	Per cent.	Per cent.	Per cent.	Per cent.	Per cent.	Per cent.
Danvers Half Long	2.30	3.68	1.55	4.08	2.51	1.03	4.00	2.82	1.00	4.14	2.96	1.16
Blanche lisse demi-longue	2.60	2.09	1.22	3.74	1.51	0.91	3.63	1.74	0.91	3.47	2.14	0.92
Jaune obtuse du Doubs	2.19	2.77	1.56	3.52	1.70	1.08	3.63	2.06	1.07	3.45	2.21	1.07
Carter's Scarlet Perfection	1.87	3.37	1.61	3.72	1.96	1.20	3.53	2.31	1.17	3.44	3.62	1.37
Rouge demi-longue du Chantenay	1.93	3.27	1.38	3.79	1.80	0.96	3.75	1.69	0.89	3.40	2.54	0.97
Carter's Summer Favorite	2.48	2.72	1.23	3.62	1.90	0.93	3.83	1.97	0.89	3.57	2.16	0.93
Carter's Early Market	1.97	3.26	1.37	3.66	2.08	1.00	3.73	1.93	0.96	3.40	2.65	1.01
Carter's Red Elephant	1.64	3.95	1.78	3.64	2.46	1.34	3.34	2.71	1.26	3.57	3.05	1.35
Blanche à collet vert	2.66	2.30	1.15	3.93	1.37	1.04	3.79	1.57	1.03	3.73	1.97	1.03

TABLE VII

COMPOSITION OF CARROTS STORED AT 32° TO 35° F.

VARIETY	AT BEGINNING OF STORAGE			AFTER 65–68 DAYS' STORAGE			AFTER 100–103 DAYS' STORAGE			AFTER 150–155 DAYS' STORAGE		
	REDUCING SUGAR	SUCROSE	ACID HYDROLYSABLE POLYSACCHARIDES	REDUCING SUGAR	SUCROSE	ACID HYDROLYSABLE POLYSACCHARIDES	REDUCING SUGAR	SUCROSE	ACID HYDROLYSABLE POLYSACCHARIDES	REDUCING SUGAR	SUCROSE	ACID HYDROLYSABLE POLYSACCHARIDES
	Per cent.	Per cent.	Per cent.	Per cent.	Per cent.	Per cent.	Per cent.	Per cent.	Per cent.	Per cent.	Per cent.	Per cent.
Danvers Half Long	0.85	3.60	1.19	1.68	2.98	1.05	1.64	2.72	0.89	1.79	2.52	0.89
Blanche lisse demi-longue	1.94	2.57	1.22	2.77	1.66	0.91	2.42	1.90	0.85	2.65	1.62	0.92
Jaune obtuse du Doubs	1.69	3.06	1.34	2.16	2.58	1.09	2.40	2.30	1.00	2.44	2.10	0.96
Carter's Scarlet Perfection	1.03	4.00	1.43	1.81	2.99	1.27	1.92	2.97	1.22	1.89	2.93	1.09
Carter's Summer Favorite	1.22	3.57	1.11	2.21	2.42	0.89	2.10	2.34	0.79	2.29	2.36	0.81
Carter's Red Elephant	0.91	4.15	1.46	2.06	3.14	1.21	2.28	2.76	1.12	1.95	2.62	1.07
Carter's Early Market	0.98	3.99	1.32	2.09	3.11	1.08	1.91	3.09	0.99	1.94	2.99	0.97
Carter's Nantes	1.15	3.67	1.19	1.80	3.10	1.01	2.10	2.81	0.92	2.21	2.56	0.86
Blanche à collet vert	1.78	3.08	1.30	2.81	1.96	1.08	2.68	2.07	1.01	2.77	1.95	0.96

TABLE VIII

SUGARS AND ACID HYDROLYSABLE POLYSACCHARIDES IN THE EQUIVALENT AT DIFFERENT STAGES OF STORAGE OF 100 GRAMS ORIGINAL WEIGHT OF CARROTS, STORED AT 39° TO 40° F.

VARIETY	AT BEGINNING OF STORAGE			AFTER 67–69 DAYS' STORAGE			AFTER 102–105 DAYS' STORAGE			AFTER 153–155 DAYS' STORAGE		
	REDUCING SUGAR	SUCROSE	ACID HYDROLYSABLE POLYSACCHARIDES	REDUCING SUGAR	SUCROSE	ACID HYDROLYSABLE POLYSACCHARIDES	REDUCING SUGAR	SUCROSE	ACID HYDROLYSABLE POLYSACCHARIDES	REDUCING SUGAR	SUCROSE	ACID HYDROLYSABLE POLYSACCHARIDES
	gm.	gm.	gm.	gm.	gm.	gm.	gm.	gm.	gm.	gm.	gm.	gm.
Danvers Half Long	2.30	3.68	1.55	3.65	2.25	0.92	3.39	2.39	0.85	3.13	2.24	0.88
Blanche lisse demi-longue	2.60	2.09	1.22	3.37	1.36	0.82	3.09	1.48	0.77	2.58	1.59	0.68
Jaune obtuse du Doubs	2.19	2.77	1.56	3.09	1.49	0.95	2.95	1.67	0.87	2.37	1.52	0.74
Carter's Scarlet Perfection	1.87	3.37	1.61	3.31	1.74	1.07	2.94	1.93	0.98	2.34	2.46	0.93
Rouge demi-longue du Chantenay	1.93	3.27	1.38	3.40	1.61	0.86	3.19	1.44	0.76	2.56	1.91	0.73
Carter's Summer Favorite	2.48	2.72	1.23	3.28	1.72	0.84	3.34	1.72	0.78	2.79	1.69	0.73
Carter's Early Market	1.97	3.26	1.37	3.35	1.90	0.92	3.23	1.67	0.83	2.62	2.05	0.78
Carter's Red Elephant	1.64	3.95	1.78	3.28	2.22	1.21	2.82	2.29	1.06	2.62	2.24	0.99
Blanche à collet vert	2.66	2.30	1.15	3.56	1.24	0.94	3.26	1.35	0.89	2.94	1.55	0.81

TABLE IX

SUGARS AND ACID HYDROLYSABLE POLYSACCHARIDES IN THE EQUIVALENT AT DIFFERENT STAGES OF STORAGE OF 100 GRAMS ORIGINAL WEIGHT OF CARROTS, STORED AT 32° TO 35° F.

VARIETY	AT BEGINNING OF STORAGE			AFTER 65–68 DAYS' STORAGE			AFTER 100–103 DAYS' STORAGE			AFTER 150–155 DAYS' STORAGE		
	REDUCING SUGAR	SUCROSE	ACID HYDROLYSABLE POLYSACCHARIDES	REDUCING SUGAR	SUCROSE	ACID HYDROLYSABLE POLYSACCHARIDES	REDUCING SUGAR	SUCROSE	ACID HYDROLYSABLE POLYSACCHARIDES	REDUCING SUGAR	SUCROSE	ACID HYDROLYSABLE POLYSACCHARIDES
	gm.	gm.	gm.	gm.	gm.	gm.	gm.	gm.	gm.	gm.	gm.	gm.
Danvers Half Long	0.85	3.60	1.19	1.61	2.86	1.01	1.55	2.56	0.84	1.68	2.37	0.84
Blanche lisse demi-longue	1.94	2.57	1.22	2.65	1.59	0.87	2.26	1.78	0.79	2.44	1.49	0.85
Jaune obtuse du Doubs	1.69	3.06	1.34	2.07	2.47	1.04	2.26	2.17	0.94	2.28	1.96	0.90
Carter's Scarlet Perfection	1.03	4.00	1.43	1.72	2.84	1.21	1.79	2.77	1.14	1.72	2.67	0.99
Carter's Summer Favorite	1.22	3.57	1.11	2.12	2.32	0.85	1.97	2.19	0.74	2.09	2.15	0.74
Carter's Red Elephant	0.91	4.15	1.46	1.97	3.01	1.16	2.14	2.60	1.05	1.85	2.49	1.02
Carter's Early Market	0.98	3.99	1.32	2.00	2.98	1.03	1.79	2.90	0.93	1.77	2.74	0.89
Carter's Nantes	1.15	3.67	1.19	1.73	2.97	0.97	1.98	2.65	0.87	2.07	2.40	0.81
Blanche à collet vert	1.78	3.08	1.30	2.71	1.89	1.04	2.53	1.95	0.95	2.59	1.83	0.90

not take into consideration the loss of water and other matter from the roots. In order to show the true changes, the actual quantities of the carbohydrates at the different stages have been calculated on the basis of 100 grams of the original material. These figures are given in tables VIII and IX.

Discussion

The ratio of reducing sugar to sucrose varies considerably with different varieties of carrots, and is not constant with single varieties in different years. Evidently seasonal effects on the state of development of the roots have greater influence than varietal characteristics. It can scarcely be said that any of the varieties is predominantly and constantly high either in sucrose or in dextrose. The two white varieties *Blanche lisse demi-longue* and *Blanche à collet vert*, which are regarded as stock carrots, show a somewhat lower sucrose content and a somewhat higher dextrose content than the orange varieties; but the pale yellow, *Jaune obtuse du Doubs*, grown extensively as a stock carrot, approaches the table carrots in sugar content.[3]

Aside from the sugars, carrots contain from about 1.25 to 1.50 per cent. of carbohydrates insoluble in 90 per cent. alcohol but easily hydrolysable by dilute acids. This material, as extraction and digestion experiments showed, consists partly of dextrin-like substances soluble in water and partly of insoluble substances hydrolysable by 0.695 N. hydrochloric acid.

The sucrose content of carrots is highest immediately after the roots have been dug and begins to decrease as soon as they have been placed in storage. The greater part of the change takes place within the first ten weeks of storage or probably within a much shorter period. Further changes in the sucrose content during the rest of the storage season are relatively small and not always in the same direction. The carrots stored at 32° to 35° F. lost, on the average, 28 per cent. of their sucrose during the first storage period and 5 and 7 per cent., respectively, during the two subsequent periods. At the higher temperature, 39° to 40° F., the average loss for all varieties during the first period was 43 per cent. of the sucrose originally present. During the next two periods there was a slight increase in sucrose in many of the varieties, resulting in an average gain of 3 and 10 per cent., respectively, for these periods. It is possible that some sucrose was reformed at this temperature.

The loss in sucrose is accompanied by a corresponding increase in reducing sugar in all varieties during the early part of the storage season. In general, there is a fair degree of proportionality between the decrease in sucrose and the increase in reducing sugar. At the higher temperature the

[3] Vilmorin-Andrieux et Cie. (Les Plantes Potagères quatrième èd. p. 71) state that it is also an excellent table carrot.

increase in reducing sugar during the first storage period is followed by a loss through respiration during the rest of the season. At the lower temperature the loss becomes insignificant, some varieties even showing a small increase in reducing sugar. Too much stress should not be laid on slight differences, however, on account of the error inherent in sampling.

The dextrins and other hydrolysable carbohydrates show an average decrease during the three storage periods of 33, 8 and 7 per cent., respectively, at the higher temperature and 20, 10 and 3 per cent. at the lower. Here, as in the case of sucrose, the transformation takes place largely during the early part of the storage season. Subsequently, there is a slight but fairly consistent decrease.

Summary

Carrots stored in cold storage rooms at a temperature of 39° to 40° F. for a period of 22 weeks lost about 26 per cent. of their weight. Those stored at 32° to 35° F. for the same length of time lost about 7 per cent. The loss consists largely of water. The loss of solid matter is equal to about one per cent. of the fresh weight of the roots.

The two principal changes which take place in carrots during storage consist in a conversion of sucrose into reducing sugar, the quantity of which is correspondingly increased; and a transformation of polysaccharides to simple sugars.

Under the conditions of the experiments here reported, these processes take place more rapidly at the higher than at the lower temperature. At the higher temperature 43 per cent. of the sucrose and 33 per cent. of the polysaccharides disappear during the first ten weeks, as compared with 28 and 20 per cent. at the lower temperature. Under constant conditions, these transformations reach a sort of equilibrium during the first ten weeks of storage or sooner. The changes thereafter are small in comparison with those taking place early during storage.

Since the flavor of carrots is determined largely by their natural content of sucrose, it is evident that for canning or for cooking the quality of the roots is highest immediately after they are dug.

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NITROGENOUS METABOLISM OF *PYRUS MALUS* L.

IV. THE EFFECT OF SODIUM NITRATE APPLICATIONS ON THE TOTAL NITROGEN AND ITS PARTITION PRODUCTS IN THE LEAVES, NEW AND ONE YEAR BRANCH GROWTH THROUGHOUT A YEAR'S CYCLE[1]

WALTER THOMAS

(WITH SEVEN FIGURES)

Introduction

The third paper of this series (**7**) gave the results of an investigation on the quantitative changes occurring in the various nitrogen fractions throughout a year's cycle in a Stayman Winesap tree, 15 years old, in the "off" year, growing in sod in the College Experimental Orchard.

Although no evidence to support the claim of certain investigators that amino-acids act as catalyzers in accelerating the rate of growth was obtained, nevertheless the results showed that amino-acids play a specific rôle. They appear to be the form in which nitrogen is carried from the roots to the metabolically active parts and, with the amides, are connected with the synthesis and utilization of proteins. Moreover, it was also shown that the unclassified or "rest" N compounds, concerning the nature of which little is known, may play a rôle as important as that of amino-acids, amines and amides, because of their apparent function in protein degradation. Finally, evidence has been produced (**8**) showing that the reduction of nitrates and the formation of amino-acids take place in this species for the most part in the fine roots; hence, a knowledge of the internal effects produced by nitrogenous fertilizers can only be obtained by following the fluctuation of the products or substances into which the NO_3 ion is transformed.

That a knowledge of the nitrogen distribution in the woody perennials is highly desirable is recognized by several investigators. Thus, PROEBSTING (**4**) has suggested that not the total nitrogen but some fraction of it might have to be considered in all attempts to correlate the relation of nutrients to the activity of the cambium. POTTER and KRAYBILL (**3**), discussing the behavior of bearing as compared to non-bearing spurs of apple trees with respect to the correlation between composition and spur performance, state

[1] Published with the approval of the Director of the Agricultural Experiment Station as scientific paper no. 432.

that the determination of the different forms of nitrogen is "a difficult or impossible task" but add "that if progress can be made in differentiating the forms of nitrogen available to spurs, it will be of greatest value in interpreting results." The present pioneer investigations have shown that the task is not impossible; it is, however, difficult, laborious, and time-consuming and will of necessity require a large force of workers in any attempt to correlate the performance of a large number of trees or plants receiving different fertilizer treatments with the forms of nitrogen available to the more metabolically active parts.

It is uncertain, with our present limited knowledge, to what extent the determination of the nitrogen distribution in plants can be applied to practical agriculture. Thus far, an insight has been obtained only into the quantitative changes taking place during a year's cycle of the water-soluble nitrogen fractions. The possibilities and limitations of nitrogen distribution investigations in this species are clearly defined, and more especially will they help to clarify all speculations relative to the utilization of any of the nitrogen fractions in the development of the carbohydrate-nitrogen relations in investigations relating to vegetative growth and flower formation.

Digressing for the moment, it may be pointed out here that the logical point of attacking such problems as, for example, the factors influencing fruit bud formation, which so many plant physiologists and horticulturists are at present attempting to solve, may be to ascertain first of all the internal conditions that cause differences in vigor, this being the external expression of the internal factors affecting the rate of metabolism, which, in turn, is limited by the rate of absorption of nutrients by the root system. The present partition results offer suggestions in this respect.

In the earlier metabolism investigations of the writer (**6**) the nitrogen partition work was carried out on one tree in the absence of the disturbing factor of developing fruit. This investigation was extended in 1924–25 to include a comparison of the course of the various nitrogen fractions throughout a year's cycle in two trees of the same variety and age growing along side one another in a homogenous soil, one of which received sodium nitrate applications and the other none. The question may be asked, what basis is there to justify a comparison of analytical results in which one tree and one tree only is compared with another of the same age and variety growing beside it in a homogenous soil? In other words, can it be postulated that the performance of the tree receiving $NaNO_3$ additions would have paralleled the untreated tree with which it is compared had the former received no such additions; or, if both trees had received the same nitrogen additions would the responses indicated by the analytical data be identical? From the results of investigations carried out by the writer in 1918–20, there appears to be little doubt that if the sampling is carried out on the

principles already stated (**7**) such comparisons as are here attempted are scientifically sound. The differences in the total nitrogen and its partition products observed in the present investigation, therefore, can logically be attributed to the addition of sodium nitrate to the one tree and not to the other.

Materials and methods

Two Stayman Winesap trees, 16 years old, growing adjacent to one another in the College Orchard, both of which had always been subjected to the same general treatment, and which resulted in very similar growth and reproductive responses, were used in this investigation. One of these trees, no. E–20, was treated with 10 pounds of sodium nitrate on April 20, 1924, just after the buds were commencing to swell, and again with another 10 pounds on June 8, 1924, at the initiation of fruit bud formation. This quantity is four times the application normally given, the object being to accentuate whatever internal differences might be produced in the nitrogen metabolism of the trees by the nitrate additions as indicated by the partition results. The external differences that existed were shown by the much darker color of the foliage and greater vegetative extension of the treated tree. Reproduction, as indicated by the yields, was 8 per cent. less on the treated tree, which would lead to the conclusion that if the theory of carbohydrate-nitrogen ratios holds, the excessive nitrate applications have tended to shift the treated trees from class III to class II in KRAUS and KRAYBILL's classification.

The collection of samples was carried out under favorable meteorological conditions in the early morning, in the manner already described (**7**). The collection was made on ten different dates between April 4, 1924, and November 11, 1924, the samples being taken to the laboratory immediately and dried in the manner already indicated (**6**).

As in the earlier experiments, no correlation could be found between the total nitrogen or any of its fractions and any of the climatic factors. However, a summary of the weather conditions is appended as a matter of record.

THE WOOD AND BARK SEPARATION PROBLEM.—In all collections dating from June 11, 1924, the wood and bark of the branch growths were separated before preserving the samples by desiccation, but, as pointed out in the third paper (**7**), it was found that no special advantage is to be derived from this procedure and that, moreover, the interpretation of the nitrogen partition results is not affected by such separations. Also, owing to the relatively small quantity of each type of branch tissues available for the partition work, it was found in many cases that there was either insufficient "wood" or "bark" to carry out satisfactorily the analytical work upon them separately. Most of the work, therefore, was carried out on samples of

TABLE I

SUMMARY OF METEOROLOGICAL DATA

DATES	MEAN MAXIMUM	MEAN MINIMUM	RAINFALL	SUNSHINE	HUMIDITY
1924	Deg. F.	Deg. F.	Inches	Per cent.	Per cent.
April 4–April 21	53.5	34.8	3.26	59.4	68.7
April 22–April 28	62.3	36.7	0.28	83.3	63.9
April 29–May 13	62.5	43.5	4.65	54.0	79.2
May 14–May 22	59.9	43.0	1.33	41.9	76.5
May 23–June 11	63.2	45.9	2.35	48.9	77.0
June 12–July 16	76.7	56.0	7.07	64.7	79.3
July 17–August 28	78.4	56.5	4.30	76.3	76.3
August 29–October 7	68.5	46.9	4.54	42.9	79.8
October 8–November 11	60.5	37.2	0.16	———	69.6

"wood" and "bark" combined in the proportions in which they were present in the original sample. There is, therefore, as far as the principal nitrogen fractions are concerned, little danger of a deficiency due to localization of the metabolically active nitrogen fractions in some tissues at the expense of others. Consequently, all analytical data have been re-calculated to the original basis as if wood and bark had been ground up together.

THE METHOD IN DETAIL.—The extractions with water were carried out in the manner outlined in the first paper (**6**).

The two fractionation schemes adopted in these metabolism investigations have already been discussed in detail (**7**). The second fractionation scheme was adopted in the present work, as fewer separations are involved, a weighty consideration where a large number of samples is to be examined, in spite of the fact that a consideration of the accuracy and limits of error as given in table III indicates that this second scheme is subject to greater analytical errors than the first scheme. However, for the present comparative studies the second scheme serves quite well.

In the non-protein filtrates from the colloidal ferric hydroxide precipitation (**6**), the following fractions were determined:—Ammonia N, amide N (asparagine and possibly glutamine N), basic N, α-mono-amino N, and also humin and melanin N from the amide and basic nitrogen determinations. The sum of these nitrogen fractions subtracted from the non-protein nitrogen gives the "rest" nitrogen.

1. *Hygroscopic water.*—This was determined on a two gram sample dried at 110° C. for 24 hours.

2. *Total water-soluble N.*—This fraction was determined in the usual way by the Kjeldahl method on 200 cc. aliquots, adopting standard methods and precautions for estimating small quantities of nitrogen.

3. *Ammonia N.*—The ammonia N was determined on a 500 cc. aliquot before treatment with colloidal iron and for reasons already given (**7**).

4. *Non-protein N.*—This was determined in the same manner as the total water-soluble nitrogen on 200 cc. aliquots of the filtrates from the colloidal ferric hydroxide precipitation.

5. *Amide N (asparagine and possibly glutamine N).*—Up to this stage the fractionation method is similar to that adopted in the earlier investigations (**7**); the point of departure is made at this stage. The determination of amide N is carried out essentially by SACHSSE's method (**5**), *i.e.*, by adding a definite quantity of HCl (sufficient to give a 4 per cent. solution) to the whole of the non-protein N filtrate remaining after the removal of the aliquots for the determination of nitrogen (which usually amounts to 1,600 cc.), and then hydrolyzing for three hours. The solution remaining after the removal of the HCl *in vacuo* is diluted to about 300 cc. with ammonia-free distilled water and distilled *in vacuo* with solid CaO (**7**). The asparagine N (with possibly some glutamine N) is obtained by subtracting the free ammonia, determined as previously indicated, from the amount obtained in this determination, *i.e.*, after hydrolysis. Proteoses were present in too small a quantity to affect the amide N results.

6. *Humin N and $Ca(OH)_2$ melanin N and basic N.*—The solution remaining in the flask from the amide N determination is filtered from the humin and $Ca(OH)_2$ melanin N, washed with hot water until free from chlorides, and the nitrogen content of these precipitates determined.

7. *The basic N.*—HAUSMANN's method (**1**) with modifications (**2, 9**) as indicated later was adopted. The filtrate and washings were concentrated *in vacuo* to about 80 cc. and then transferred to a beaker, the contents being cooled to 20° C. Afterwards 2.5 cc. concentrated H_2SO_4 were slowly added, followed by a solution containing 20 gm. of phosphotungstic acid and 5 gm. concentrated H_2SO_4 per 100 cc., the mixture being added drop by drop. Six cc. of this phosphotungstic acid mixture were quite sufficient in the present work. Care is to be taken to avoid more than a slight excess of the phosphotungstic acid solution; otherwise difficulty will be encountered in the subsequent amino-acid determination, owing to precipitation of some of the phosphotungstic acid. The solutions were diluted to 200 cc., heated on the water bath until the precipitates of the bases were dissolved, and then allowed to stand for two or three days. The granular precipitate was filtered off and washed with a small quantity of phosphotungstic acid solution containing 2.5 gm. phosphotungstic acid and 5 gm. concentrated H_2SO_4 per 100 cc. The technique of washing these precipitates was carried out in accordance with OSBORNE and HARRIS's (**2**) recommendations. VAN SLYKE (**9**) gives additional observations on the care in precipitating and washing

TABLE II

THE PERCENTAGES OF IMBIBITIONAL AND TOTAL WATER

ONE YEAR BRANCH GROWTH (TREE NO. E–20)

SERIES NO.	COLLECTING DATE	DESCRIPTION	FRESH WEIGHT	MOISTURE FREE WEIGHT	IMBIBITIONAL WATER	TOTAL WATER
			gm.	gm.	Per cent.	Per cent.
76	April 4, 1924	(wood and bark)	70.0	35.7	49.0	58.1
77	April 21, 1924	(wood and bark)	121.0	58.1	52.0	59.0
92	April 28, 1924	(wood and bark)	55.0	27.0	50.9	56.9
114	May 13, 1924	(wood and bark)	73.0	35.0	52.0	58.4
142	May 22, 1924	(wood and bark)	51.0	22.0	56.9	57.8
161	June 11, 1924	(wood)	55.5	28.8	48.1	51.7
162	June 11, 1924	(bark)	43.8	17.9	59.1	60.7
185A	July 16, 1924	(wood)	78.8	43.7	44.4	46.8
185B	July 16, 1924	(bark)	56.6	22.6	58.8	61.8
196	August 28, 1924	(wood)	70.1	39.5	43.7	46.9
196A	August 28, 1924	(bark)	52.6	22.6	57.0	60.9
203A	October 7, 1924	(wood)	88.3	55.6	37.0	45.0
203B	October 7, 1924	(bark)	63.1	28.2	55.3	57.3
237	November 11, 1924	(wood)	123.0	71.1	42.2	44.7
238	November 11, 1924	(bark)	78.4	38.4	50.8	54.0

ONE YEAR BRANCH GROWTH (TREE NO. E–22)

SERIES NO.	COLLECTING DATE	DESCRIPTION	FRESH WEIGHT	MOISTURE FREE WEIGHT	IMBIBITIONAL WATER	TOTAL WATER
			gm.	gm.	Per cent.	Per cent.
60	April 4, 1924	(wood and bark)	69.0	35.0	49.3	53.0
68	April 21, 1924	(wood and bark)	118.0	56.0	52.5	56.2
86A	April 28, 1924	(wood and bark)	69.0	34.0	50.7	60.0
105	May 13, 1924	(wood and bark)	81.0	37.0	54.3	60.4
124	May 22, 1924	(wood and bark)	53.3	21.6	59.4	60.0
154	June 11, 1924	(wood)	52.5	33.0	37.7	42.9
155	June 11, 1924	(bark)	54.0	24.3	55.0	60.2
180A	July 16, 1924	(wood)	72.4	41.0	43.6	47.8
180B	July 16, 1924	(bark)	51.8	21.4	56.4	60.9
199A	August 28, 1924	(wood)	71.2	40.4	43.2	44.2
199B	August 28, 1924	(bark)	53.6	26.7	47.5	58.7
215A	October 7, 1924	(wood)	90.6	54.4	40.0	43.1
215B	October 7, 1924	(bark)	65.0	36.5	56.2	57.4
218	November 11, 1924	(wood)	120.0	42.4	35.4	40.6
217	November 11, 1924	(bark)	75.0	33.7	52.2	57.0

TABLE II (*Continued*)

THE PERCENTAGES OF IMBIBITIONAL AND TOTAL WATER

NEW GROWTH (TREE NO. E–20)

SERIES NO.	COLLECTING DATE	DESCRIPTION	FRESH WEIGHT	MOISTURE FREE WEIGHT	IMBIBITIONAL WATER	TOTAL WATER
			gm.	gm.	Per cent.	Per cent.
160	June 11, 1924		50.0	14.4	66.2	71.2
185	July 16, 1924	(wood)	32.0	12.0	62.5	65.4
186	July 16, 1924	(bark)	46.0	16.0	65.2	68.1
191	August 28, 1924	(wood)	30.0	16.0	46.6	50.2
192	August 28, 1924	(bark)	31.0	14.0	54.8	57.9
206	October 7, 1924	(wood)	52.3	31.0	40.7	44.1
207	October 7, 1924	(bark)	66.6	21.5	67.7	69.8
217	November 11, 1924	(wood)	68.0	42.5	37.5	43.9
218	November 11, 1924	(bark)	51.0	31.2	38.8	44.5

NEW GROWTH (TREE NO. E–22)

SERIES NO.	COLLECTING DATE	DESCRIPTION	FRESH WEIGHT	MOISTURE FREE WEIGHT	IMBIBITIONAL WATER	WATER TOTAL
			gm.	gm.	Per cent.	Per cent.
153	June 11, 1924		64.0	20.8	67.5	72.9
188A	July 16, 1924	(wood)	31.6	12.3	60.8	64.2
189	July 16, 1924	(bark)	46.7	17.7	61.8	65.6
196A	August 28, 1924	(wood)	37.0	21.0	43.2	47.3
196	August 28, 1924	(bark)	40.0	19.0	52.5	54.2
212	October 7, 1924	(wood)	46.9	29.0	38.0	42.0
211	October 7, 1924	(bark)	40.5	21.1	48.1	51.6
230	November 11, 1924	(wood)	62.6	38.0	37.7	41.9
231	November 11, 1924	(bark)	54.2	27.6	49.1	52.6

these phosphotungstic acid precipitates. However, no special difficulty was encountered in washing them. Since the precipitates were small, care had to be exercised to employ small filters and to avoid more than two or three washings, using suction and a wash solution cooled to 0° C. In this respect the technique differs from that employed in protein work, in which the quantities of basic nitrogen obtained from 2 to 3 gm. of protein are comparatively large.

Nitrogen was determined in these precipitates in the usual way, the precipitate and filter being transferred to the Kjeldahl flash direct, since it was not necessary to decompose the basic phosphotungstic precipitates, inas-

TABLE II (*Concluded*)

THE PERCENTAGES OF IMBIBITIONAL AND TOTAL WATER

LEAVES (TREE NO. E–20)

SERIES NO.	COLLECTING DATE	FRESH WEIGHT	MOISTURE FREE WEIGHT	IMBIBITIONAL WATER	TOTAL WATER
		gm.	gm.	Per cent.	Per cent.
138	May 13, 1924	50.0	13.0	74.0	77.0
140	May 22, 1924	70.3	20.0	72.6	76.4
166	June 11, 1924	97.0	32.0	67.0	70.0
189	July 16, 1924	204.0	82.0	59.8	62.6
195	August 28, 1924	144.0	60.0	58.3	60.7
210	October 7, 1924	170.0	77.0	54.7	57.9
222A	November 11, 1924	40.0	19.3	51.8	56.3

LEAVES (TREE NO. E–22)

SERIES NO.	COLLECTING DATE	FRESH WEIGHT	MOISTURE FREE WEIGHT	IMBIBITIONAL WATER	TOTAL WATER
		gm.	gm.	Per cent.	Per cent.
106	May 13, 1924	49.0	16.0	66.7	72.3
129	May 22, 1924	73.3	18.0	75.4	77.0
153A	June 11, 1924	85.0	24.3	60.4	65.6
180	July 16, 1924	203.0	82.0	59.6	65.6
199	August 28, 1924	214.0	89.0	58.4	61.4
215	October 7, 1924	168.0	63.5	62.2	65.2
233A	November 11, 1924	38.0	15.5	59.2	64.3

much as the determination of the diamino acids—histidine, arginine and lysine—was not under consideration in this investigation.

8. *α-Mono-amino N.*—The filtrate and washings from the phosphotungstic acid precipitation were made alkaline (pH 7.5) with 50 per cent. NaOH and then acid (pH 6.0) with acetic acid, concentrated *in vacuo* to about 80 cc., *i.e.*, to the point at which salts begin to separate out, and finally made up to a definite volume (usually 100 cc.). The α-mono-amino N was determined in the usual way in the VAN SLYKE micro-apparatus.

9. *"Rest" N.*—This was calculated by difference, as already described.

Experimental results

A description of the samples with their fresh and moisture free weights is given in table II, and the partition results in table III.

TABLE III

TABLE III

TOTAL NITROGEN AND TOTAL WATER-SOLUBLE NITROGEN IN THE NEW (1924) BRANCH GROWTH, THE ONE YEAR (1923) BRANCH GROWTH AND LEAVES, TOGETHER WITH THE DISTRIBUTION OF THE NON-PROTEIN CONSTITUENTS EXPRESSED AS PERCENTAGES OF FRESH AND MOISTURE FREE WEIGHTS, RESPECTIVELY

ONE YEAR BRANCH GROWTH (TREE NO. E–20)

SERIES NO.	COLLECTING DATE	TOTAL N	TOTAL WATER-SOLUBLE N	TOTAL NON-PROTEIN N	AMMONIA N	AMINO N	AMIDE N	BASIC N	HUMIN N	RESIDUAL N
				As percentages of fresh weight of material						
76	April 4, 1924	0.263	0.0370	0.0317	0.0059	0.0041	0.0029	0.0046	0.0015	0.0126
77	April 21, 1924	0.308	0.0463	0.0380	0.0005	0.0070	0.0094	0.0057	0.0028	0.0197
92	April 28, 1924	0.329	0.0698	0.0541	0.0022	0.0084	0.0086	0.0108	0.0028	0.0216
114	May 13, 1924	0.225	0.0582	0.0458	0.0012	0.0064	0.0042	0.0083	0.0027	0.0229
142	May 22, 1924	0.287	0.0789	0.0429	0.0019	0.0120	0.0080	0.0096	0.0029	0.0084
161										
162	June 11, 1924	0.248	0.0443	0.0349	0.0013	0.0035	0.0049	0.0066	0.0026	0.0159
185A										
185B	July 16, 1924	0.310	0.0733	0.0455	0.0014	0.0113	0.0127	0.0042	0.0035	0.0122
196	August 28, 1924	0.274	0.0643	0.0531	0.0020	0.0070	0.0110	0.0107	0.0021	0.0201
203A										
203B	October 7, 1924	0.298	0.0697	0.0598	0.0017	0.0080	0.0134	0.0099	0.0027	0.0239
237										
238	November 11, 1924	0.346	0.0852	0.0716	0.0020	0.0130	0.0140	0.0110	0.0030	0.0275
				As percentages of moisture free weight of material						
76	April 4, 1924	0.628	0.0835	0.0756	0.0140	0.0098	0.0070	0.0110	0.0035	0.0303
77	April 21, 1924	0.750	0.1130	0.0929	0.0012	0.0170	0.0230	0.0140	0.0068	0.0481
92	April 28, 1924	0.764	0.1620	0.1256	0.0050	0.0195	0.0200	0.0250	0.0066	0.0500
114	May 13, 1924	0.540	0.1400	0.1101	0.0030	0.0155	0.0100	0.0200	0.0066	0.0550
142	May 22, 1924	0.680	0.1870	0.1017	0.0045	0.0285	0.0190	0.0228	0.0069	0.0200
161										
162	June 11, 1924	0.560	0.1000	0.0788	0.0030	0.0080	0.0110	0.0150	0.0058	0.0360
185A										
185B	July 16, 1924	0.660	0.1560	0.0968	0.0030	0.0240	0.0270	0.0090	0.0075	0.0260
196	August 28, 1924	0.545	0.1280	0.1057	0.0040	0.0140	0.0220	0.0214	0.0042	0.0400
203A										
203B	October 7, 1924	0.600	0.1400	0.1200	0.0035	0.0160	0.0270	0.0200	0.0055	0.0480
237										
238	November 11, 1924	0.690	0.1700	0.1430	0.0040	0.0260	0.0280	0.0220	0.0060	0.0550

TABLE III (*Continued*)

TOTAL NITROGEN AND TOTAL WATER-SOLUBLE NITROGEN IN THE NEW (1924) BRANCH GROWTH, THE ONE YEAR (1923) BRANCH GROWTH AND LEAVES, TOGETHER WITH THE DISTRIBUTION OF THE NON-PROTEIN CONSTITUENTS EXPRESSED AS PERCENTAGES OF FRESH AND MOISTURE FREE WEIGHTS, RESPECTIVELY

ONE YEAR BRANCH GROWTH (TREE NO. E–22)

SERIES NO.	COLLECTING DATE	TOTAL N	TOTAL WATER-SOLUBLE N	TOTAL NON-PROTEIN N	AMMONIA N	AMINO N	AMIDE N	BASIC N	HUMIN N	RESIDUAL N
				As percentages of fresh weight of material						
60	April 4, 1924	0.299	0.0384	0.0286	0.0066	0.0045	0.0034	0.0047	0.0010	0.0086
68	April 21, 1924	0.279	0.0520	0.0389	0.0026	0.0068	0.0057	0.0064	0.0013	0.0160
86A	April 28, 1924	0.288	0.0560	0.0395	0.0022	0.0076	0.0047	0.0058	0.0032	0.0160
105	May 13, 1924	0.166	0.0416	0.0309	0.0016	0.0031	0.0014	0.0041	0.0030	0.0168
124	May 22, 1924	0.186	0.0385	0.0375	.....	.....	.....	.....	.....	.....
154										
155	June 11, 1924	0.171	0.0353	0.0290	0.0015	0.0013	0.0027	0.0030	0.0036	0.0169
180A										
180B	July 16, 1924	0.153	0.0387	0.0257	0.0016	0.0025	0.0025	0.0027	0.0036	0.0128
199A										
199B	August 28, 1924	0.158	0.0321	0.0211	0.0017	0.0015	0.0048	0.0032	0.0025	0.0075
215A										
215B	October 7, 1924	0.151	0.0357	0.0262	0.0014	0.0028	0.0061	0.0034	0.0029	0.0096
228										
229	November 11, 1924	0.208	0.0432	0.0352	0.0019	0.0045	0.0071	0.0040	0.0024	0.0152
				As percentages of moisture free weight of material						
60	April 4, 1924	0.635	0.0818	0.0608	0.0140	0.0095	0.0072	0.0100	0.0021	0.0182
68	April 21, 1924	0.638	0.1187	0.0887	0.0060	0.0155	0.0130	0.0147	0.0030	0.0365
86A	April 28, 1924	0.720	0.1400	0.0988	0.0055	0.0190	0.0118	0.0145	0.0080	0.0400
105	May 13, 1924	0.480	0.1203	0.0893	0.0046	0.0090	0.0040	0.0118	0.0088	0.0485
124	May 22, 1924	0.465	0.1051	0.0865	0.0040	0.0120	0.0085	0.0165	0.0080	0.0400
154										
155	June 11, 1924	0.405	0.0832	0.0687	0.0035	0.0030	0.0065	0.0072	0.0085	0.0400
180A										
180B	July 16, 1924	0.340	0.0860	0.0570	0.0035	0.0055	0.0055	0.0060	0.0080	0.0285
199A										
199B	August 28, 1924	0.315	0.0640	0.0422	0.0034	0.0030	0.0095	0.0063	0.0050	0.0150
215A										
215B	October 7, 1924	0.314	0.0740	0.0543	0.0030	0.0058	0.0126	0.0070	0.0060	0.0200
228										
229	November 11, 1924	0.437	0.0910	0.0740	0.0040	0.0095	0.0150	0.0085	0.0050	0.0320

229	November 11, 1924	0.437	0.0910	0.0740	0.0040	0.0095	0.0150	0.0085	0.0050	0.0320

TABLE III (*Continued*)

TOTAL NITROGEN AND TOTAL WATER-SOLUBLE NITROGEN IN THE NEW (1924) BRANCH GROWTH, THE ONE YEAR (1923) BRANCH GROWTH AND LEAVES, TOGETHER WITH THE DISTRIBUTION OF THE NON-PROTEIN CONSTITUENTS EXPRESSED AS PERCENTAGES OF FRESH AND MOISTURE FREE WEIGHTS, RESPECTIVELY

NEW GROWTH (TREE NO. E–20)

SERIES NO.	COLLECTING DATE	TOTAL N	TOTAL WATER-SOLUBLE N	TOTAL NON-PROTEIN N	AMMONIA N	AMINO N	AMIDE N	BASIC N	HUMIN N	RESIDUAL N
			As percentages of fresh weight of material							
160	June 11, 1924	0.432	0.0490	0.0369	0.0013	0.0124	0.0078	0.0046	0.0013	0.0092
185										
186	July 16, 1924	0.363	0.0818	0.0714	0.0035	0.0250	0.0089	0.0073	0.0035	0.0228
191										
192	August 28, 1924	0.378	0.0771	0.0716	0.0010	0.0207	0.0128	0.0096	0.0034	0.0246
206										
207	October 7, 1924	0.424	0.0979	0.0810	0.0031	0.0265	0.0148	0.0069	0.0032	0.0278
217										
218	November 11, 1924	0.517	0.1114	0.0964	0.0023	0.0281	0.0201	0.0046	0.0040	0.0373
			As percentages of moisture free weight of material							
160	June 11, 1924	1.503	0.1700	0.1290	0.0045	0.0430	0.0270	0.0160	0.0045	0.0320
185										
186	July 16, 1924	0.843	0.1900	0.1650	0.0082	0.0580	0.0206	0.0170	0.0082	0.0530
191										
192	August 28, 1924	0.784	0.1600	0.1485	0.0020	0.0430	0.0265	0.0200	0.0070	0.0510
206										
207	October 7, 1924	0.802	0.1850	0.1533	0.0058	0.0500	0.0280	0.0130	0.0060	0.0525
217										
218	November 11, 1924	0.900	0.1940	0.1680	0.0040	0.0490	0.0350	0.0080	0.0070	0.0650

TABLE III (*Continued*)

TOTAL NITROGEN AND TOTAL WATER-SOLUBLE NITROGEN IN THE NEW (1924) BRANCH GROWTH, THE ONE YEAR (1923) BRANCH GROWTH AND LEAVES, TOGETHER WITH THE DISTRIBUTION OF THE NON-PROTEIN CONSTITUENTS EXPRESSED AS PERCENTAGES OF FRESH AND MOISTURE FREE WEIGHTS, RESPECTIVELY

NEW GROWTH (TREE NO. E–22)

SERIES NO.	COLLECTING DATE	TOTAL N	TOTAL WATER-SOLUBLE N	TOTAL NON-PROTEIN N	AMMONIA N	AMINO N	AMIDE N	BASIC N	HUMIN N	RESIDUAL N
		As percentages of fresh weight of material								
153	June 11, 1924	0.379	0.0278	0.0215	0.0012	0.0063	0.0033	0.0028	0.0023	0.0057
181										
182	July 16, 1924	0.199	0.0378	0.0309	0.0013	0.0079	0.0031	0.0046	0.0015	0.0125
196A										
196B	August 28, 1924	0.227	0.0414	0.0330	0.0019	0.0062	0.0041	0.0036	0.0027	0.0145
211										
212	October 7, 1924	0.276	0.0500	0.0377	0.0017	0.0062	0.0070	0.0037	0.0040	0.0193
230										
231	November 11, 1924	0.300	0.0527	0.0462	0.0013	0.0073	0.0087	0.0031	0.0029	0.0213
		As percentages of moisture free weight of material								
153	June 11, 1924	1.400	0.1026	0.0703	0.0043	0.0230	0.0120	0.0102	0.0086	0.0212
181										
182	July 16, 1924	0.605	0.1150	0.0940	0.0040	0.0240	0.0095	0.0140	0.0045	0.0380
196A										
196B	August 28, 1924	0.547	0.1000	0.0798	0.0045	0.0150	0.0100	0.0088	0.0065	0.0350
211										
212	October 7, 1924	0.557	0.1010	0.0762	0.0035	0.0125	0.0142	0.0075	0.0080	0.0390
230										
231	November 11, 1924	0.603	0.1089	0.0954	0.0030	0.0150	0.0180	0.0064	0.0060	0.0440

TABLE III (*Continued*)

TOTAL NITROGEN AND TOTAL WATER-SOLUBLE NITROGEN IN THE NEW (1924) BRANCH GROWTH, THE ONE YEAR (1923) BRANCH GROWTH AND LEAVES, TOGETHER WITH THE DISTRIBUTION OF THE NON-PROTEIN CONSTITUENTS EXPRESSED AS PERCENTAGES OF FRESH AND MOISTURE FREE WEIGHTS, RESPECTIVELY

LEAVES (TREE NO. E–22)

SERIES NO.	COLLECTING DATE	TOTAL N	TOTAL WATER-SOLUBLE N	TOTAL NON-PROTEIN N	AMMONIA N	AMINO N	AMIDE N	BASIC N	HUMIN N	RESIDUAL N
		As percentages of fresh weight of material								
138	May 13, 1924	0.830	0.0690	0.0575	0.0019	0.0092	0.0028	0.0120	0.0081	0.0219
140	May 22, 1924	0.806	0.0802	0.0658	0.0015	0.0145	0.0042	0.0165	0.0078	0.0217
166	June 11, 1924	0.774	0.0732	0.0660	0.0012	0.0138	0.0027	0.0165	0.0063	0.0219
189	July 16, 1924	0.804	0.1058	0.0954	0.0022	0.0204	0.0026	0.0224	0.0120	0.0370
195	August 28, 1924	0.817	0.1014	0.0880	0.0016	0.0137	0.0076	0.0157	0.0110	0.0385
210	October 7, 1924	0.777	0.0973	0.0869	None	0.0147	0.0084	0.0177	0.0164	0.0379
222A	November 11, 1924	0.677	0.0961	0.0861	None	0.0125	0.0118	0.0122	0.0059	0.0433
		As percentages of moisture free weight of material								
138	May 13, 1924	3.610	0.3000	0.2500	0.0085	0.0402	0.0121	0.0520	0.0350	0.0950
140	May 22, 1924	3.415	0.3400	0.2790	0.0065	0.0615	0.0180	0.0700	0.0330	0.0920
166	June 11, 1924	2.210	0.2440	0.2200	0.0040	0.0465	0.0092	0.0550	0.0310	0.0730
189	July 16, 1924	2.150	0.2830	0.2550	0.0058	0.0545	0.0070	0.0600	0.0320	0.0990
195	August 28, 1924	2.080	0.2580	0.2240	0.0040	0.0350	0.0194	0.0400	0.0280	0.0980
210	October 7, 1924	1.845	0.2310	0.2060	None	0.0350	0.0200	0.0420	0.0390	0.0900
222A	November 11, 1924	1.550	0.2200	0.1970	None	0.0285	0.0270	0.0280	0.0135	0.0990

TABLE III (*Concluded*)

TOTAL NITROGEN AND TOTAL WATER-SOLUBLE NITROGEN IN THE NEW (1924) BRANCH GROWTH, THE ONE YEAR (1923) BRANCH GROWTH AND LEAVES, TOGETHER WITH THE DISTRIBUTION OF THE NON-PROTEIN CONSTITUENTS EXPRESSED AS PERCENTAGES OF FRESH AND MOISTURE FREE WEIGHTS, RESPECTIVELY

LEAVES (TREE NO. E–20)

SERIES NO.	COLLECTING DATE	TOTAL N	TOTAL WATER-SOLUBLE N	TOTAL NON-PROTEIN N	AMMONIA N	AMINO N	AMIDE N	BASIC N	HUMIN N	RESIDUAL N
		As percentages of fresh weight of material								
106	May 13, 1924	0.860	0.0637	0.0526	0.0015	0.0076	0.0028	0.0122	0.0097	0.0188
129	May 22, 1924	0.782	0.0584	0.0478	0.0012	0.0100	0.0043	0.0150	0.0078	0.0097
153A	June 11, 1924	0.681	0.0578	0.0530	0.0019	0.0096	0.0028	0.0148	0.0103	0.0135
180	July 16, 1924	0.663	0.0612	0.0563	0.0014	0.0107	0.0014	0.0213	0.0096	0.0119
199	August 28, 1924	0.672	0.0571	0.0510	None	0.0097	0.0039	0.0135	0.0112	0.0127
215	October 7, 1924	0.543	0.0539	0.0480	None	0.0090	0.0052	0.0139	0.0099	0.0080
233A	November 11, 1924	0.443	0.0493	0.0437	None	0.0068	0.0071	0.0082	0.0107	0.0190
		As percentages of moisture free weight of material								
106	May 13, 1924	3.520	0.2300	0.1900	0.0055	0.0275	0.0101	0.0440	0.0350	0.0677
129	May 22, 1924	3.400	0.2540	0.2080	0.0050	0.0435	0.0185	0.0650	0.0340	0.0420
153A	June 11, 1924	1.980	0.1680	0.1540	0.0055	0.0280	0.0082	0.0430	0.0300	0.0393
180	July 16, 1924	1.926	0.1780	0.1637	0.0040	0.0312	0.0040	0.0620	0.0280	0.0345
199	August 28, 1924	1.740	0.1480	0.1320	None	0.0250	0.0100	0.0350	0.0290	0.0330
215	October 7, 1924	1.560	0.1550	0.1320	None	0.0260	0.0150	0.0400	0.0285	0.0230
233A	November 11, 1924	1.240	0.1380	0.1225	None	0.0190	0.0200	0.0230	0.0300	0.0305

Accuracy and limits of error

Table IV gives the differences between duplicate determinations in certain of the series calculated as percentages of the mean readings.

TABLE IV

Differences obtained in certain duplicate determinations

Series no.	Total N	Total water-soluble N	α-Mono-amino N	Amide N
	Per cent.	Per cent.	Per cent.	Per cent.
60	0.48	1.60	6.75	7.80
76	.....	1.20	7.20	3.00
114	0.40	1.30	4.60	7.10
129	0.14	.....	5.80	1.00
138	0.30	1.20	3.90	4.50

The total nitrogen shows a maximum difference of 0.48 per cent.; the water-soluble nitrogen of 1.60 per cent.; the α-mono-amino nitrogen of 7.20 per cent.; and the amide nitrogen of 7.80 per cent. All variations in the metabolism figures, therefore, can be regarded as significant.

Discussion and conclusions

Total nitrogen

Total nitrogen in the one year branch growth.—It was pointed out in the third paper (7) that during the first growth of spring translocation of nitrogen from the one and two year branches to the young shoots is very marked. The effect of this demand is strikingly illustrated in the present investigation, as shown in fig. 1. The total nitrogen of the one year growth of the untreated tree drops during the period from April 28 to May 13 from 0.288 per cent. to 0.166 per cent. on the fresh weight basis, *i.e.*, from 0.720 per cent. to 0.480 per cent. on the moisture free basis. On May 13 the leaves contain as high as 0.860 per cent. nitrogen on the fresh weight basis and 3.520 per cent. on the moisture free basis.

The effect of the application of 10 pounds of $NaNO_3$ on April 20 to tree no. E–20 is distinctly evident in both the quantitative and qualitative relationships. After May 13, when the trees are in full bloom, the total nitrogen curves of the two trees take a different course. Basing observations on the moisture free basis (dy/dt), *i.e.*, the slope of the total nitrogen curves of the untreated tree is negative to the end of September; whereas in the treated tree (dy/dt), though fluctuating considerably, shows a net change of almost zero. In other words, from May 13 to the end of September there has been

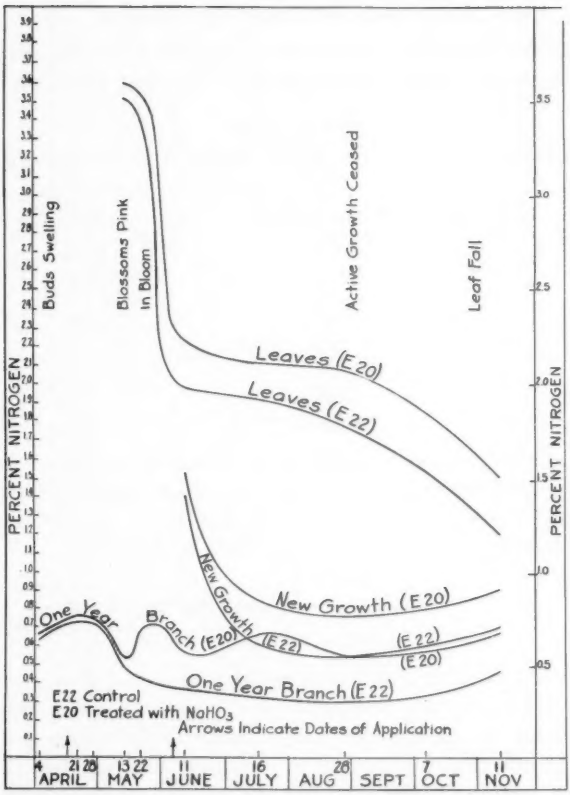

FIG. 1. Total nitrogen in the leaves, new (1924) branch growth and one year (1923) branch growth.

no net gain or loss in total nitrogen in the one year (1923) branch growth of the treated tree; whereas in the unfertilized tree the total nitrogen of the one year (1923) branch growth decreased 35 per cent. during the same period.

The increase in the total nitrogen content of the one year branch growth of tree no. E–20 continues until it is utilized by the demands of the developing shoots. An inspection of the curves shows that the second application (June 8) of 10 pounds of $NaNO_3$ to E–20 is absorbed more rapidly than the first application. These facts lend further support to the suggestion that the rate of absorption of a nutritive element is fundamental and probably one of the most important factors in soil fertility and plant nutrition problems.

The differences in total nitrogen content as a result of fertilization are again clearly indicated at the period when the trees enter the rest period. On April 4 the one year branch growth of the untreated tree has 0.299 per cent. nitrogen, but on November 11 only 0.208 per cent. on the fresh weight basis, *i.e.*, 0.635 per cent. and 0.437 per cent., respectively, on the moisture free basis; whereas the treated tree has 0.263 per cent. and 0.346 per cent., *i.e.*, 0.628 per cent. and 0.690 per cent. on the fresh and moisture free basis, respectively, on the respective dates. The results are clearly shown in table V.

TABLE V

COMPARISON OF THE PERCENTAGES OF TOTAL NITROGEN OF THE ONE YEAR BRANCH GROWTHS OF EACH TREE AT CERTAIN PERIODS

COLLECTING DATE	UNTREATED		TREATED	
	Fresh weight basis	Moisture free basis	Fresh weight basis	Moisture free basis
April 4, 1924	0.299	0.635	0.263	0.628
April 28, 1924	0.288	0.720	0.329	0.764
November 11, 1924	0.208	0.437	0.346	0.690

TOTAL NITROGEN OF THE NEW (1924) BRANCH GROWTH.—The differences in total nitrogen content of the new (1924) branch growth are also quite marked on the two trees. The untreated tree had a total nitrogen content of 0.379 per cent. on June 11 and of 0.300 per cent. on November 11 on the fresh weight basis, *i.e.*, 1.40 per cent. and 0.603 per cent., respectively, on the moisture free basis; whereas the treated tree had a total nitrogen content of 0.432 per cent. and 0.517 per cent. on the fresh weight basis, *i.e.*, 1.503 per cent. and 0.900 per cent., respectively, on the moisture free basis on these dates.

Table VI conveniently shows the effect of the $NaNO_3$ applications on the total nitrogen content of the new growth.

It has already been shown in the third paper that the results of applying 5 pounds of nitrogen are only just about sufficient to maintain nitrogen equilibrium under the conditions of that experiment. Considering, then, only the season's (1924) branch growth and the one year (1923) branch growth, the present results show that whereas the untreated tree will start the next season on a much lower nitrogen plane than in the previous season, the treated tree has slightly more than maintained equilibrium. This appears to be in accordance with the practical results obtained in this Experiment Station, indicating that 10-pound applications of $NaNO_3$ per tree may be practicable economically.

TABLE VI

COMPARISON OF THE PERCENTAGES OF TOTAL NITROGEN OF THE NEW (1924) BRANCH GROWTHS OF EACH TREE AT CERTAIN PERIODS

COLLECTING DATE	UNTREATED		TREATED	
	Fresh weight basis	Moisture free basis	Fresh weight basis	Moisture free basis
June 11, 1924	0.379	1.400	0.432	1.503
November 11, 1924	0.300	0.604	0.517	0.900

TOTAL NITROGEN IN THE LEAVES.—Table VII shows the effect of fertilization on the total nitrogen content of the leaves. The effect on the leaves is more clearly indicated in the diminution in concentration of nitrogen as indicated by the much lower figures for the untreated tree on the fresh weight basis than in the absolute quantities.

TABLE VII

COMPARISON OF THE PERCENTAGES OF TOTAL NITROGEN OF THE LEAVES OF EACH TREE AT CERTAIN PERIODS

COLLECTING DATE	UNTREATED		TREATED	
	Fresh weight basis	Moisture free basis	Fresh weight basis	Moisture free basis
May 13, 1924	0.860	3.520	0.830	3.610
November 11, 1924	0.443	1.240	0.677	1.550

Although the character of the curves is the same, there are decided quantitative differences. On May 13 the total nitrogen content of the young leaves of the untreated tree is 0.860 per cent. and of the treated tree 0.830 per cent. (fresh weight basis), or 3.520 per cent. and 3.610 per cent. (moisture free basis), respectively. At the end of the period of chlorophyll degeneration, about the time of leaf fall, the untreated tree contained 0.443 per cent. and the treated tree 0.677 per cent. (fresh weight basis), or 1.240 per cent. and 1.550 per cent. (moisture free basis), respectively.

The same fall in total nitrogen content of the leaves from the time of bud opening to the middle of June, observed in the previous investigation and described in the third paper of the series, takes place in both trees. This corresponds to the period of most rapid growth.

THE NITROGEN DISTRIBUTION

THE PARTITION OF NITROGEN IN THE LEAVES.—The marked differences in the nitrogen metabolism of the two trees is very evident from an inspection

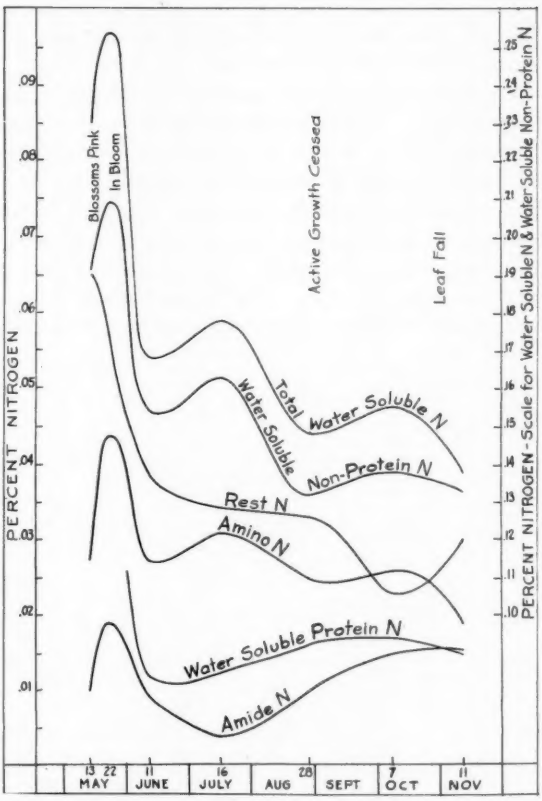

FIG. 2. Total water-soluble N, water-soluble protein N, non-protein N, amino N, amide N and "rest" N in the leaves of the untreated tree (no. E–22).

of figs. 2 and 3. The analytical results are given in table III. The most striking differences are in the total water-soluble nitrogen, the non-protein nitrogen, the amino nitrogen, and the "rest" nitrogen. The effect of the second application of $NaNO_3$ on June 8 results in a large increase of total water-soluble and non-protein nitrogen. Thus, in the treated tree the increase in concentration (as indicated by the fresh weight results) of water-soluble N and non-protein N from June 11 to July 16 amounts to 43 and 16 per cent., respectively, but during the same period the total water-soluble and non-protein nitrogen of the leaves of the untreated tree show an increase of only 6 and 8 per cent., respectively. The earlier investigations show a similar fluctuation, occurring, however, a month later. That the increase at

this period in the untreated tree can be due solely to increased nitrification of the soil is quite clear, for fig. 4 shows that the one year (1923) branch growth increases in total water-soluble nitrogen also.

The course followed by the amino and amide nitrogen in the leaves calls for no special comment. The curves are similar to those described in the earlier investigations (**7**). It is to be noted that they are much higher in the fertilized tree as a result of the $NaNO_3$ treatment.

The "rest" nitrogen compounds represent, both in absolute magnitude and in their metabolic gyrations, an extremely important group in that the evidence indicates that these unclassified compounds form an important link in the degradation of proteins (**7**). The fluctuations of this fraction in the two trees show more remarkable differences than any of the other fractions.

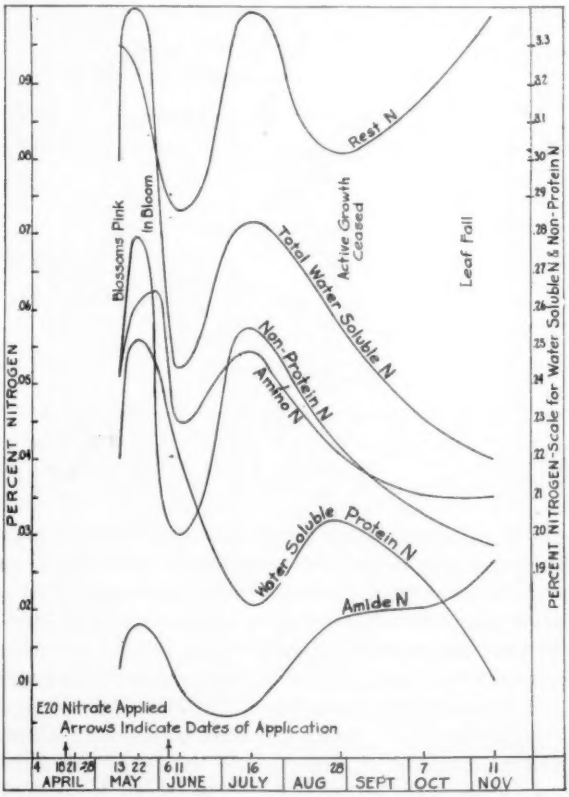

FIG. 3. Total water-soluble N, water-soluble protein N, non-protein N, amino N, amide N and "rest" N in the leaves of the treated tree (no. E–20).

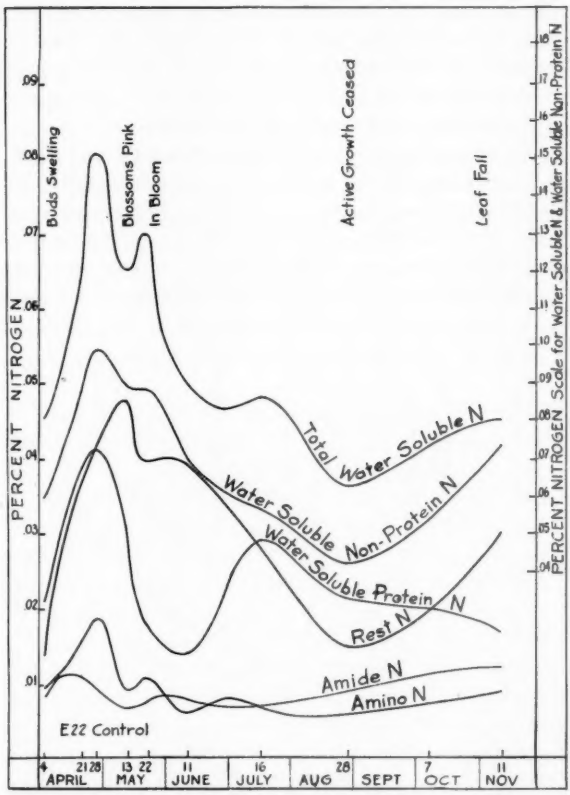

FIG. 4. Total water-soluble N, water-soluble protein N, non-protein N, amino N, amide N and "rest" N in the one year (1923) branch growth of the untreated tree (no. E–22).

The positive slope of the "rest" nitrogen curve in fig. 3 just after the application of nitrate to the treated tree on June 8 is a characteristic of this tree only; the leaves of the untreated tree do not show it. It is hoped to be able to isolate and identify some of these unclassified nitrogen compounds later.

THE PARTITION OF NITROGEN IN THE ONE YEAR (1923) BRANCH GROWTH.—The conclusions drawn in the earlier investigations are, in general, confirmed in this investigation; and it will, therefore, be unnecessary to discuss them in detail again. The visual presentation by means of figs. 4 and 5 shows very clearly the differences between the treated and untreated trees.

The influence of the sodium nitrate addition on the nitrogen partition on the one year (1923) branch growth of tree no. E–20 on April 20 and

June 8 is very marked. From a comparison of the cycle curves (figs. 4 and 5) it is readily seen that the period of absorption is characterized by a marked increase in the total water-soluble and the non-protein nitrogen with concomitant increases in the amino, amide and basic nitrogen of tree E–20 (treated) in comparison with tree E–22 (untreated).

On April 21 the percentages of total water-soluble nitrogen in the one year (1923) branch growth of trees E–20 (treated) and E–22 (untreated) are 0.0463 and 0.0520 per cent., respectively, but three weeks later the quantities present are 0.0789 and 0.0385 per cent., respectively—an increase of 85 per cent. and a decrease of 30 per cent., respectively. Again, at the last sampling, November 11, tree no. E–20 (treated) has 0.0852 per cent.,

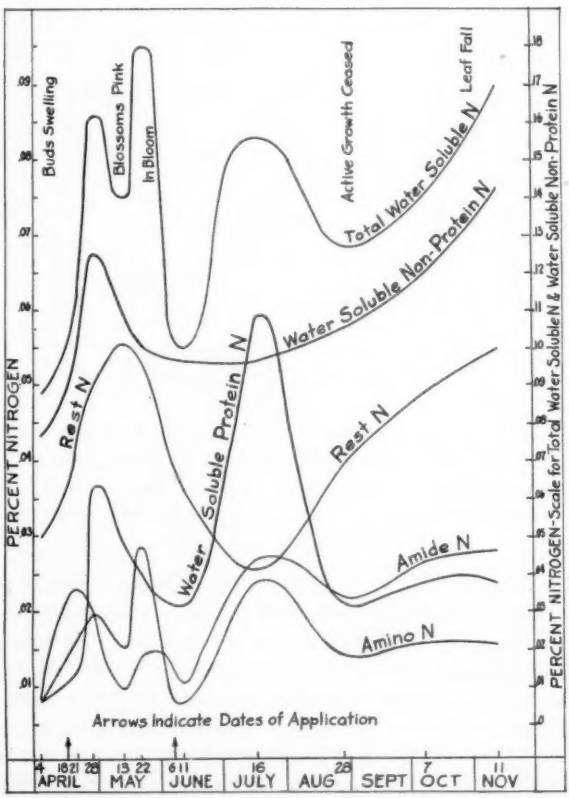

FIG. 5. Total water-soluble N, water-soluble protein N, non-protein N, amino N, amide N and "rest" N in the one year (1923) branch growth of the treated tree (no. E–20).

but tree no. E–22 (untreated) has only 0.0432 per cent. of water-soluble nitrogen. The non-protein nitrogen results of the trees parallel these figures, *viz.*, 0.0380 and 0.0389 per cent. on April 21 and 0.0429 and 0.0375 per cent. three weeks later.

The mono-amino and amide nitrogen curves, too, are interesting. Up to May 10 (about three weeks after the first application of $NaNO_3$ to E–20) the curves for amino N show little differences in the two trees. About this date, however, the mono-amino and amide nitrogen begin to increase rapidly in the treated tree. The same phenomena are observed after the second application of $NaNO_3$ on June 8. As already pointed out, at this time of the year absorption of the $NaNO_3$ and its transformation to $-NH_2$ and $-CO.NH_2$ groups is more rapid, for the effect is noticeable in the leaves, one year branch growth and new growth within ten days of the second application.

The importance of the "rest" nitrogen compounds both in magnitude and in their metabolic gyrations is again clearly indicated in the one year (1923) branch growth. Their significance is more or less a mystery, but an inspection of the graphs will readily confirm the conclusions previously drawn (**7**) where it was suggested that they must take part in the degradation of proteins.

THE PARTITION OF NITROGEN IN THE NEW (1924) BRANCH GROWTH.—The differences in the nitrogen partition in the new (1924) branch growth are more marked than in the one year (1923) branch growth. The behavior of the treated tree (E–20) with respect to the total water-soluble and non-protein nitrogen cycle corresponds to the results obtained in the earlier investigations (**7**). The main point of interest is the steep positive slope as shown in fig. 7, about a week after the second application of $NaNO_3$ (June 8), and the very large increase again in these constituents during the fall storage of nitrogen.

A complete interpretation of the total water-soluble and non-protein nitrogen cycles (fig. 6) of the untreated tree is more difficult. It is apparent that the concentrations of nitrogen in both fractions are much lower in the check tree than in the nitrogen treated tree and that these differences are associated with differences in vigor.

The mono-amino acid and amide nitrogen cycles in the new (1924) branch growth of the two trees show the characteristic differences already observed in the one year (1923) branch growth, *e.g.*, the effect of the second application of $NaNO_3$ on June 8 to tree E–20 is very evident from the marked increase in amino N and the "rest" N compounds. The large storage as amide N in the new branch growth as a result of fertilization is very marked; thus, on November 11 the amide nitrogen in E–20 is 0.0201 per cent. and 0.0350 per cent., but in E–22 only 0.0087 per cent. and 0.0180 per

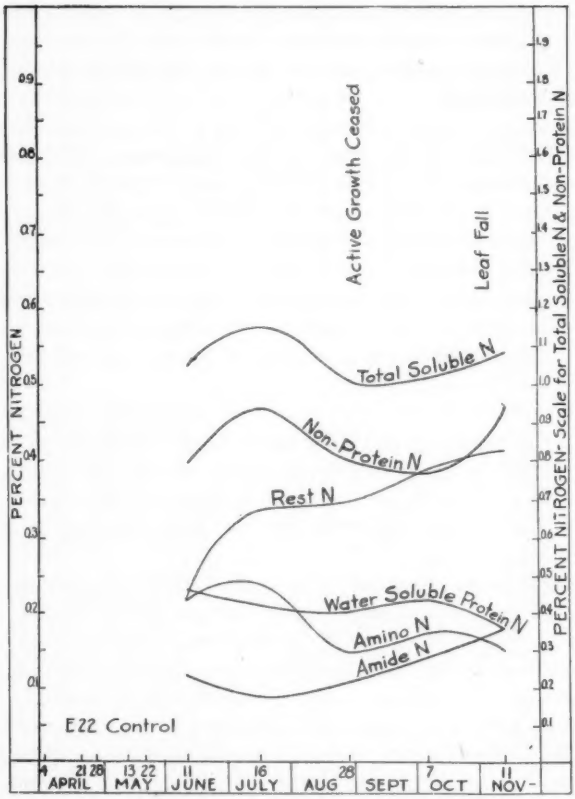

FIG. 6. Total water-soluble N, water-soluble protein N, non-protein N, amino N, amide N and "rest" N in the new (1924) branch growth of the untreated tree (no. E–22).

cent. on the fresh and moisture free basis, respectively, and the mono-amino N 0.0281 per cent. and 0.0490 per cent. and 0.0073 per cent. and 0.0150 per cent. on the fresh and moisture free basis, respectively.

Summary and conclusions

These studies are an extension of the partition investigations reported in the third paper of the series (7) to two Stayman Winesap trees of the same age, growing on sod in a homogenous soil. One of these trees was fertilized with $NaNO_3$ and the other left unfertilized. In general, the present findings confirm the conclusions drawn from the previous studies.

The present investigations have shown that:

1. The total nitrogen of the leaves and shoot growth vary with growth. During the early growth of spring the demand of the young shoots upon the nitrogen of the one year (1923) branch growth is very marked. This is especially shown in the untreated tree, E–22.
2. The effect of the two 10-pound applications of sodium nitrate on April 20, 1924, and June 8, 1924, on the total nitrogen content of the two trees may be thus summarized:
   (a) The total nitrogen content of the one year (1923) branch growth of the fertilized tree from the period of full bloom to the end of September increased 10 per cent., but the total nitrogen of the

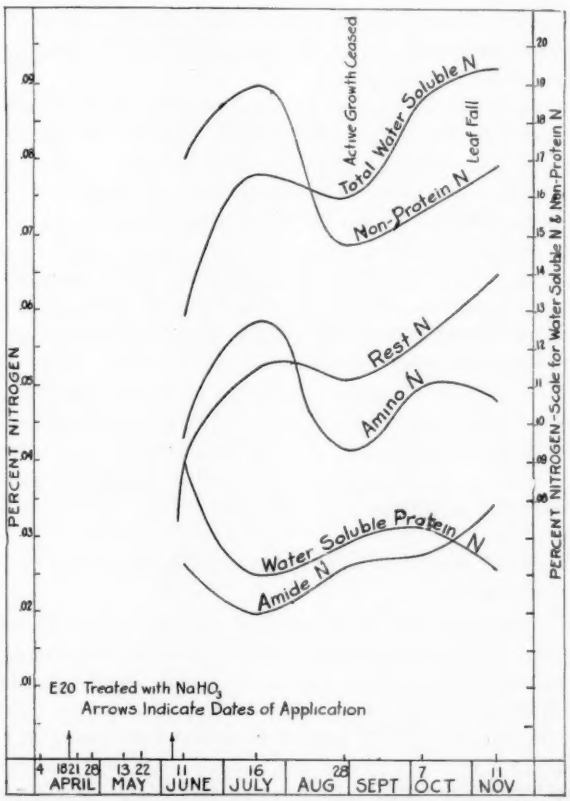

FIG. 7. Total water-soluble N, water-soluble protein N, non-protein N, amino N, amide N and "rest" N in the new (1924) branch growth of the treated tree (no. E–20).

one year (1923) branch growth of the unfertilized tree decreased 35 per cent. during this period.

(b) From June 11 to November 11 the total nitrogen of the new growth of the treated tree (E–20) decreased 40 per cent., but that of the untreated tree (E–22) decreased 57 per cent.

(c) The leaves of the untreated tree are consistently lower than in the treated tree.

A much lower plane of nitrogen metabolism is thus indicated in the unfertilized tree.

3. The period of most rapid absorption of the NO_3 ion is characterized by a large increase in the total water-soluble and non-protein nitrogen, concomitant with which there is an increase in the amino, amide, basic, and "rest" nitrogen fractions of the fertilized as compared with the unfertilized tree in the leaves, the one year (1923) branch growth and the new (1924) branch growth.
4. From the course of the curves for total water-soluble and non-protein nitrogen or amino N of the fertilized as compared with the unfertilized tree, it can be deduced that the first application of sodium nitrate on April 20 took three weeks to reach the one year branches. The same curves show that the absorption and translocation of the NO_3 ions of the second application on June 6 is much more rapid, taking about one week only to get to the more metabolically active parts of the tree.
5. The total water-soluble nitrogen and non-protein nitrogen is decidedly higher in the metabolically active parts of the treated tree as compared with the untreated tree throughout the whole cycle. These differences correspond with the relative vigor of the trees and must be associated with differences in enzymatic activity. Taken in conjunction with the close parallelism of the amino-acid fraction with the total water-soluble and non-protein fractions (7), these findings suggest a possible simplification of the nitrogen distribution problem in practical horticulture.
6. Terminal growth was decidedly greater in the fertilized than in the unfertilized tree.
7. Nitrogen is stored in the fall as amide N and as "rest" N compounds. The storage of these compounds is much higher in the treated tree than in the untreated tree. This fact is of extreme importance in connection with the demands of the young shoots in the spring. This is true of both the one year (1923) branch growth and the new (1924) branch growth.
8. The "rest" nitrogen compounds form an important connecting link in the synthesis and degradation of proteins.

The writer desires to thank Mr. C. A. KERN for the determinations of the hygroscopic and imbibitional water and Miss ETHEL GINGRICH for assistance in calculating and checking the computations.

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RELATION OF COMPOSITION TO GROWTH AND FRUITFULNESS OF YOUNG APPLE TREES AS AFFECTED BY GIRDLING, SHADING, AND PHOTOPERIOD[1]

R. H. ROBERTS

(WITH TWENTY-TWO FIGURES)

Introduction

An effort is being made to record the relations of internal composition of apple trees to their vegetative responses, especially to the particular growth characters accompanying blossom bud formation. Various environmental conditions are being used to vary the plant composition. In a previous report (**4**) the effects of nitrogen fertilization were considered. It was observed that the vegetative condition as measured by such characters as length of growth, leaf colors, bark colors, and plant composition varied directly with the nitrogen fertilization under the cultural conditions provided: dwarf trees in pots in the greenhouse. On the other hand, blossom bud formation was not correlated to cultural treatment, but rather to growth character and to a "balance" in composition when the carbohydrate and nitrogen content are compared. This is a condition similar to that described by KRAUS and KRAYBILL for fruit development in the tomato (**2**). The most vegetative plants or growths were highest in nitrogen, lowest in carbohydrates, and unfruitful; the least vegetative plants were lowest in nitrogen, highest in carbohydrates and were unfruitful; and the moderately vegetative plants were intermediate in nitrogen and carbohydrate content and were fruitful.

The significance of the above relation of composition to blossom bud formation and fruiting lies in this: if reproduction is related to growth character, which is the response to internal composition, rather than directly to cultural treatment, then, (1) we are provided with a basis for interpreting the conflict of results secured from the limiting factor or plot method of investigating tree performance, and (2) we can use the growth character of the tree as the basis for diagnosing orchard conditions when attempting to decide upon needed cultural treatments. Instead of using the present unsuccessful method of trying to duplicate a cultural treatment which is reported as being profitable in another locality, the practical grower

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can attempt to produce by any feasible means and with a reasonable assurance of success, such amounts and types of growth as give the desired fruiting responses. To illustrate, the use of readily available nitrogen fertilizers has become very general in recent years, because of the frequent increase in fruiting which results. In situations where low production is due to an over-vegetative type of growth, nitrogen fertilization tends further to delay or reduce production. A similar condition pertains as regards pruning and cultivation; these may be harmful as well as beneficial to fruiting, depending upon the vegetative condition of the trees being considered.

Materials and methods

The data presented herein were collected to determine the effect of three other environmental conditions upon tree composition, blossom bud formation, and to further test whether or not fruitfulness was related directly to the treatment or to the balance in composition produced by the treatments. The conditions used were girdling, shading, and reduced photoperiod.

As regards shading and girdling, one hundred yearling Wealthy trees grafted on standard seedling stocks were planted in early May, 1926. When the new growth averaged one to two inches long, one half of the row was covered with a burlap shade on a lath frame. Further, one half of the shaded and of the unshaded trees were girdled by removing a half inch of bark about mid-height. The girdle was covered with grafting wax. Several girdled trees died. Many girdled-and-shaded trees died. The trouble was obviously due to lack of root development. No trees were lost from girdling in late July after appreciable root development had occurred. Some sucker growth was produced below the girdles. A few trees produced sufficient callus to bridge the girdle late in the growing season. These latter were avoided when sampling for chemical analyses. The effect of shading is probably less than would ordinarily obtain, as the sunshine from June to November was only 41.5 per cent. of possible or 69.4 per cent. of normal in 1926.

Results

The growth produced under the different treatments is shown in table I. The growth data were obtained from averages of paired trees. That is, the "check" is made up of trees having a previous season length and weight like the treated trees. Thus each treatment has a separate check. There is considerable difference in checks because the girdled trees which died were smaller than the ones which lived and because the larger check trees tended to grow more than the smaller ones.

There are some items of especial interest in table I:

1. The greatest amount or quantity of growth when measured by per cent. gain in weight of tree, total length of shoots, or amount of new roots was made

TABLE I

EFFECT OF SHADE AND GIRDLING UPON GROWTH OF YOUNG APPLE TREES

TREATMENT	INCREASE IN WEIGHT	LENGTH TERMINAL BRANCH	DIAMETER TERMINAL GROWTH	INTERNODE LENGTH	TOTAL SHOOTS		RATIO TOTAL SHOOTS TO PER CENT. WEIGHT INCREASE	GREEN WEIGHT OF NEW ROOTS[2]
					NUMBER	LENGTH		
	Per cent.	cm.	mm.	mm.		cm.		gm.
Sun, ungirdled	162.9	37.2	2.86	24.5	3.35	97.0	0.60	7.43
Shade, ungirdled	51.4	40.4	2.51	30.0	2.93	85.2	1.66	2.67
check	135.6	32.6	2.69	23.6	2.93	71.3	0.53	
Sun, girdled	40.6	12.1	3.40	20.7	3.66	52.2	1.29	2.48
check	154.5	40.9	2.68	23.1	3.00	86.3	0.56	
Shade, girdled	19.6	21.2	3.08	27.4	3.20	49.2[1]	2.51	0.49
check	168.5	36.7	3.02	25.8	3.40	104.3	0.62	

[1] Less branching as well as less growth of suckers than sun and girdling.
[2] Average of three trees.

by the ungirdled sun trees. The least was made by the girdled, shaded trees.

2. On the other hand, the extremes of kind or quality of growth when measured by such characters as individual shoot length, diameter and internode distance are found in the shaded trees and the girdled trees in the light.
3. The differences in character of growth above and below the girdles, as will be pointed out later, make an error in measuring total growth of tree. For instance, the seasonal increase in weight of shaded and girdled trees (average 10.8 gm.) is less in some cases than the increase above the girdle, thus showing a loss of weight below the girdle.

The failure to differentiate between the amount and character or quality of growth is believed to be a frequent error in interpreting tree growth data secured from cultural plots.

Further details of growth character as affected by the shading and girdling are presented in tables II and III and figs. 1 to 19.

Some responses of particular interest, most of which are recorded in the tables or illustrations follow:

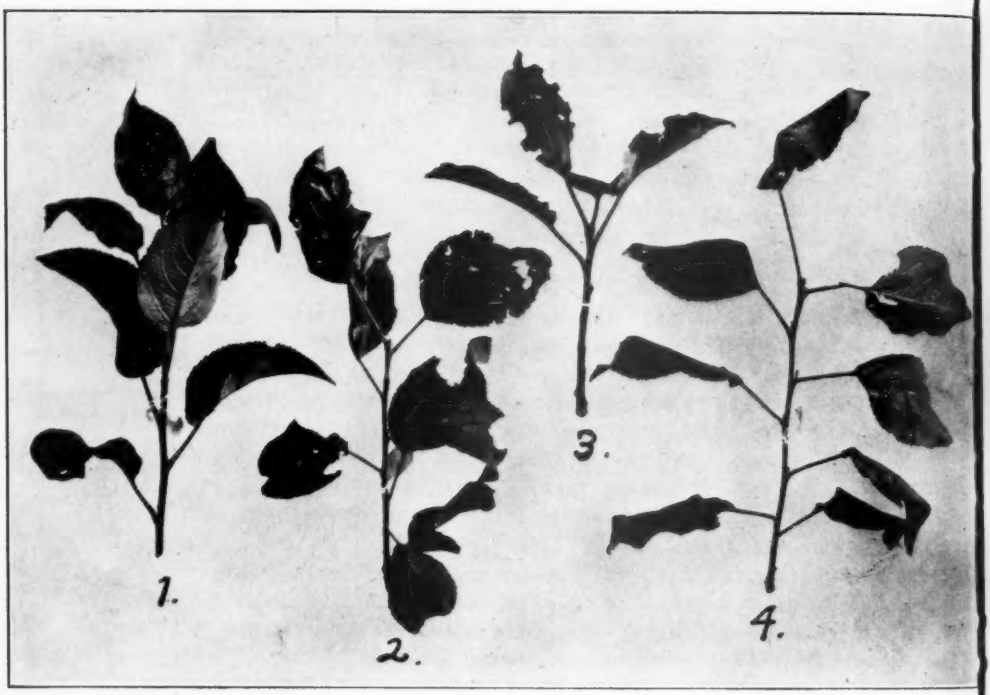

FIG. 1. Character of shoot and foliage growth: Left to right, 1. Sun; 2. Shade; 3. Sun and girdled; 4. Shade and girdled. The leaves of 1 and 4 are very similar in texture although appearing different due to a difference in orientation.

TABLE II

EFFECT OF SHADE AND GIRDLING UPON FOLIAGE DEVELOPMENT OF YOUNG APPLE TREES*

TREATMENT	LEAF SIZE	LEAF THICKNESS		CELLS PER SQ. MM.		STOMATA PER SQ. MM.	MILLION CELLS PER LEAF SURFACE	
		ABOVE GIRDLE	BELOW GIRDLE	UPPER SURFACE	LOWER SURFACE		LOWER	UPPER
	sq. in.	mm.	mm.					
Sun	4.08	0.276	0.272	1737	1920	583	4.57	5.06
Shade	6.12	0.153	0.161	1385	1895	460	5.46	7.46
Sun, girdled	2.18	0.289	0.255	2158	2772	820	3.03	3.90
Shade, girdled	5.00	0.158	0.145	1961	2468	580	6.32	7.72

* Details of leaf and stem structure recorded by MARIAN DEATS ABEGG.

TABLE III

EFFECT OF SHADE AND GIRDLING UPON STEM ANATOMY IN YOUNG APPLE TREES

Treatment	New growth									New xylem		
	Radius			Ratio			Number cells in radii			Trunk		Roots
	Pith	Xylem	Bark*	Pith / Xylem	Pith / Bark	Xylem / Bark	Phloem	Rays	Xylem	Upper	Lower	
	mm.	mm.	mm.							mm.	mm.	mm.
Sun	0.622	0.306	0.610	2.03	1.02	0.50	15	8	18	0.68	0.74	0.63
Shade	0.585	0.263	0.479	2.22	1.22	0.55	11	7	13	0.23	0.38	0.24
Sun, girdled	0.620	0.502	0.722	1.23	0.86	0.70	18	18	39	0.81	0.34	0.16
Shade, girdled	0.577	0.381	0.565	1.51	1.02	0.67	13	9	20	0.30	0.07	0.04

* Phloem and cortex.

1. The influence of the treatments upon diameter growth.
2. The marked reduction in secondary thickening in the shade and below girdles. Bast fibre development was also nearly absent. Only the check trees had second year bast in the lower trunks.
3. Ray cell shapes varied greatly as well as vessel, wood-fibre, and wood-parenchyma cell sizes.
4. Starch grain sizes and storage cell wall thickenings were markedly affected.
5. Leaf palisade was strikingly reduced by shade.
6. Large leaves on shade trees resulted from both larger cells and greater number per leaf.
7. Girdling and shading greatly reduced root development. This is correlated with the effect upon new xylem production.

Notes:

1. Three trees were used for each sample.
2. A modified Kjeldahl was not used in determining total nitrogen, as apple wood is rarely found to contain an appreciable amount of nitrates.
3. Ptyalin digestion was used in extracting starch.
4. The reducing substances were obtained by the Munson-Walker method and were measured by the Shaffer-Hartman titration method. The volumetric method gives results with mature apple wood comparable to the gravimetric determinations.
5. Acid hydrolysis was accomplished by refluxing with 2.5 per cent. sulphuric acid for one hour.
6. The bark was not analyzed separate from the wood. This may be a source of error. In general, however, it seems that bark and wood analyses vary more in percentages, due obviously to a greater inert fraction in the wood, than in the direction of difference. While they may show decided differences in percentage the fluctuations in amounts are parallel.

Table IV shows the usual lack of relation between the starch fraction and either the reducing or acid hydrolyzable fractions. As usual, these latter are not correlated with vegetative condition or anatomical structure.

The relatively high starch content below the girdles in unshaded trees is obviously due to slight utilization of reserves, the small top growth made before the time of girdling being from material near the growing points and from newly synthesized foods.

The intermediate carbohydrate content and nitrogen carbohydrate relation of new shoots on sun and shade-and-girdled trees as compared to the shaded and sun-and-girdled trees is in agreement with their intermediate growth character.

The low nitrogen content of the wood and roots below the girdles, especially in the shade, was a surprising condition. The growth character of the suckers arising from this region were of a characteristic "high nitrogen" appearance. As there is a greater reduction in carbohydrate percentage than in nitrogen percentage below the girdles, the lower portion of the stems do have a "high nitrogen" condition. This is taken as evidence that growth

TABLE IV

EFFECT OF SHADE AND GIRDLING UPON COMPOSITION OF YOUNG APPLE TREES

SAMPLE	TREATMENT	MOISTURE	SPECIFIC GRAVITY	TOTAL N	STARCH	REDUCING SUBSTANCE		ACID HYDROLYZABLE	TOTAL CARBOHYDRATE	RATIO TO N	
						FREE	TOTAL			STARCH	TOTAL CARBOHYDRATE
		Per cent.		Per cent.	Per cent.	Per cent.	Per cent.	Per cent.	Per cent.		
One year wood	Sun	52.1	1.115	0.876	7.40	3.84	6.53	6.05	19.98	8.45	23.12
	Shade	54.9	1.084	0.869	6.61	3.37	7.76	3.73	18.10	7.61	21.08
	Sun, girdled	50.2	1.356	0.775	9.92	4.13	8.28	7.65	25.85	13.15	33.40
	Shade, girdled	51.3	1.207	0.953	8.88	3.50	7.48	4.31	20.67	9.32	21.70
Two year wood, upper half	Sun	46.3	1.106	0.557	5.82	3.57	5.14	4.08	15.04	10.46	27.10
	Shade	48.1	1.008	0.668	5.07	3.46	4.92	4.66	14.65	7.58	21.95
	Sun, girdled	46.6	1.056	0.540	8.29	3.89	6.28	6.48	21.05	15.36	38.99
	Shade, girdled	48.3	1.009	0.582	7.40	5.52	7.75	5.48	20.63	12.72	35.50
Two year wood, lower[1] half	Sun	44.7	1.005	0.528	4.97	1.81	3.13	2.99	11.09	9.40	21.05
	Shade	47.1	1.042	0.526	4.47	2.26	3.23	4.38	12.08	8.49	23.00
	Sun, girdled	48.6	1.010	0.443	4.57	1.80	3.50	4.04	12.11	10.32	27.35
	Shade, girdled	52.6	0.961	0.376	0.92	1.47	2.97	2.96	6.85	2.45	18.23
Two year roots	Sun	55.2	1.049	0.813	14.20	4.13	6.11	4.29	24.60	17.50	30.30
	Shade	56.8	1.002	0.963	12.42	4.16	6.32	5.66	24.40	12.95	25.38
	Sun, girdled	55.9	0.983	0.757	15.98	4.22	6.71	5.54	28.23	21.12	37.30
	Shade, girdled	59.2	0.977	0.718	4.02	4.93	8.42	6.32	18.76	5.59	26.12

[1] Below girdle in case of girdled trees.

character is determined more by the relations or qualities of foods than by the actual quantities present.

The low nitrogen content below the girdles may be associated with the poor root development on girdled trees rather than being a result of girdling, although the nitrogen content of the top is not related to root extension either on a percentage or absolute basis.

TABLE V

NITROGEN AND STARCH IN YOUNG APPLE TREES*

TREATMENT		NEW GROWTH	TWO YEAR WOOD		TWO YEAR ROOTS
			UPPER HALF	LOWER HALF	
		mg.	mg.	mg.	mg.
Nitrogen	Sun	19.64	22.73	17.98	19.00
	Shade	11.36	32.68	30.18	23.45
	Sun, girdled	13.86	21.60	20.32	19.75
	Shade, girdled	16.75	29.30	8.98	8.68
Starch	Sun	165.7	237.8	169.2	332.1
	Shade	86.7	247.6	256.2	337.5
	Sun, girdled	177.1	331.9	209.6	417.0
	Shade, girdled	156.1	373.0	21.2	48.7

* The crown piece, including some stem and the main root as well as the new fibrous roots, are not included. Differences in previous season's bulk make direct comparisons of treatments useless, but the general tendencies where chemical differences are large can be clearly seen.

TABLE VI

EFFECT OF SHADE AND GIRDLING UPON THE PERCENTAGE OF BLOSSOM BUD FORMATION IN YOUNG APPLE TREES

TREATMENT	SPURS	TERMINALS	LATERALS	TOTAL BUDS
Sun	0.0	0.0	0.0	0.0
Shade	0.0	0.0	0.0	0.0
Sun, girdled	47.8	100.0	65.7	65.2
Shade, girdled	1.2	5.6	0.0	0.8

Two conditions will be noted from table VI. One is the relation of blossom bud formation to composition and growth character, and not directly to treatment. That is, girdled trees in the sun produced abundant blossom buds, but when such trees are shaded they are almost unfruitful as are the sun, ungirdled trees which have a like composition and growth character.

It will also be noted that blossom bud formation accompanied a condition of least growth and greatest carbohydrate content. This is because the very high carbohydrate, under-vegetative type of tree was not represented in the present series. The blossoming of trees of intermediate or "balanced" composition will be found in the following report upon the effect of reduced photoperiod upon apple tree performance.

It was desired to determine whether a "short day" had a direct effect upon blossom bud formation or produced its effects through changes in the plant composition. Consequently, trees which were in very different vegetative conditions due to previous variations in nitrogen nutrition were grown with both a full and a six-hour photoperiod from March to August, 1925. The trees were grown in the greenhouse in 12-inch earthen pots resting in trays for sub-irrigating. A ventilated "cage" constructed of slats and building paper was placed over the short day trees from 2 P. M. until 8 A. M. each day. Only six trees were grown in each lot. Because of the pronounced and consistent behavior of these trees (fig. 20), a record of their performance is given here.

The chemical composition on August 6, is shown together with the blossoming observed in early 1926, in table VII. Sampling was at approximate maturity of the long day plants. The short day trees with a high nitrogen nutrient definitely stopped vegetative extension only when water was withheld. They gave no sign of "maturing" as did the other trees.

There was a marked increase in length of growth. Variations in leaf and stem anatomy were striking. These will be reported in detail after repeating the photoperiod experiment with a larger number of trees.

Very clearly, a short photoperiod did not have the same blossom-forming effect upon the two lots of trees: it induced blossoming of the low nitrogen trees but entirely prevented blossom bud formation on the high nitrogen trees. Reproduction is here obviously related to the chemical composition rather than to the treatment and is certainly not directly induced by either a short or a long day. With a well balanced nutrition, apple trees are apparently "long day" plants, that is, they form blossom buds during the long days of early summer.

The long day-low nitrogen trees could be classed if desired as high carbohydrate, under-vegetative trees, and the short day-high nitrogen trees as low carbohydrate, over-vegetative trees. In a simliar way the short day-low nitrogen and long day-high nitrogen trees could be spoken of as "balanced," fruitful trees. That is, fruitfulness accompanied a condition of intermediate amount of growth as well as nitrogen and carbohydrate content.

The behavior of the trees in a second season is shown by figs. 21 and 22. The removal of samples for analysis constituted a heavy pruning. The

TABLE VII

EFFECT OF PHOTOPERIOD UPON CHEMICAL COMPOSITION AND BLOSSOM BUD FORMATION IN DWARF APPLE TREES*

SAMPLE	DAY LENGTH	NITROGEN NUTRIENT	MOISTURE	TOTAL N	STARCH	REDUCING SUBSTANCES		ACID HYDROLYZABLE	TOTAL CARBOHYDRATE	RATIO TO N		BLOSSOMS
						FREE	TOTAL			STARCH	CARBOHYDRATE	
			Per cent.	Per cent.	Per cent.	Per cent.	Per cent.	Per cent.	Per cent.			Per cent.
New growth	Long	Low[1]	46.1	0.70	8.08	2.43	5.25	7.01	20.34	11.5	29.1	0.0
	Short	Low	52.1	1.07	5.56	2.51	5.53	6.08	17.17	5.2	16.0	16.5
	Long	High	50.3	0.87	6.17	1.61	3.87	6.23	16.27	7.1	18.7	14.3
	Short	High	62.9	1.17	2.81	1.67	3.57	4.22	10.60	2.4	9.1	0.0
Two year wood	Long	Low	48.5	0.43	10.91	2.96	4.94	7.81	23.66	25.4	55.0	
	Short	Low	50.2	0.55	5.74	1.78	4.83	6.86	17.43	14.4	31.7	
	Long	High	48.0	0.38	5.37	1.42	2.20	3.98	11.55	14.1	30.4	
	Short	High	51.0	0.60	3.04	1.52	2.94	4.73	10.71	5.1	17.8	

* Analyses by N. MOGENDORFF.

[1] Traces of NO_3 in tap water used. Other elements were supplied to the quartz sand used as soil.

effect of this operation upon new growth was noticeably less pronounced than the influence of light environment. Attention is especially called to fig. 21, showing the growth made by trees practically without nitrogen in the nutrient for four or five seasons. Two years of short-day treatment has produced a slender type of growth with large thin leaves which is characteristic of over-vegetative trees. That is, reduced period of illumination gave a type of growth wholly comparable to making heavy applications of nitrate to trees with full illumination. The nitrogen-fed trees in the short day for two years produced partially chlorotic foliage and an extremely weak spindly growth.

Before proceeding to a further discussion of the chemical and growth data, it is well to inquire as to what significance the analyses have. What value should be placed upon the chemical data?

The reducing substances extracted in hot concentrated alcohol usually show little relation to vegetative condition. This might well be expected in view of its presence being dependent upon variations in light and temperature as well as upon utilization.

Starch is the one carbohydrate being analyzed for which does usually appear to be closely related to growth condition. Starch values are obviously high in some samples as has already been pointed out (**4**). Very probably boiling the sample to gelatinize the starch extracts some reducing substance. Consequently the range in variation of starch content is really larger than is indicated by analyses.

A discouraging fact is the failure to secure correlation between the acid hydrolyzable fraction and vegetative condition. Miss BRADBURY (**1**) has pointed out that this is probably because the wall thickenings which are present in apple tree tissues and function as reserves are not hydrolyzed in the course of standard analysis. Because of the marked relation between vegetative condition and anatomical development, the "hemicellulose" fraction should be expected to show a clear correlation to plant growth. Here the analyses clearly do not indicate as large differences as exist between samples.

There is a large discrepancy in the amount of material extracted and that recovered as reducing substances. This is not only large but it is also variable, ranging from approximately 30 to 60 per cent. in the samples reported upon in table IV. This difference can not be explained by clarification precipitates, as clarification increased the reducing power of more than a majority of the 20 samples compared, table VIII.

Discussion

From these and other observations it is hardly necessary to say that a mathematical ratio between materials as nitrogen and the carbohydrate

TABLE VIII

EFFECT OF CLARIFICATION UPON THE PERCENTAGES OF STARCH AND ACID HYDROLYZABLE CONSTITUENTS OF SOME APPLE WOOD SAMPLES

SAMPLE	STARCH		ACID HYDROLYZABLE	
	CLARIFIED	UNCLARIFIED	CLARIFIED	UNCLARIFIED
One year wood. Check	7.40	7.12	6.05	3.81
Shade	6.61	5.74	3.73	4.21
Girdled	9.92	10.71	7.65	5.62
Girdled and shade	8.88	9.99	4.31	4.85
Two year wood. Check	5.82	4.26		
Shade	5.07	4.28		
Girdled	8.29	6.18		
Girdled and shade	7.40	6.82		
Two year roots. Check	14.20	15.88	4.29	3.77
Shade	12.42	11.74	5.66	4.78
Girdled	15.98	12.85	5.54	5.30
Girdled and shade	4.02	4.02	6.32	5.88

fractions is only an indication of relations. It might almost be asked if careful histological and micro-chemical records of qualitative differences would not better indicate the course of metabolism than do the macro-analyses when present standard methods are followed. Better methods of analyzing for the usual substances are needed as well as methods for other substances than are now considered.

Rather than suggesting that chemical data are of little importance because they show that different samples are not much different in composition, it seems to be a better interpretation to say that chemical data are often not of much value or significance because they represent only a part, too often small, of the real chemical differences existing between the samples, as is indicated by their marked differences in anatomical and growth character.

In addition to indications that blossom bud formation is related to a condition of "balanced" growth and composition, there is another significant situation which appeared in both series of trees. This is the relation of carbohydrate content or carbohydrate-nitrogen relation to vegetative elongation. This is shown particularly by the long growth of the short-day trees, especially those with a low nitrogen nutrient (fig. 21) and by the short growth of the girdled trees in the field.

How do very poorly vegetative trees become strongly vegetative by being placed in a short photoperiod? The change in percentage of nitrogen appears directly related to the fall in specific gravity due to a reduced carbohydrate content. With the respiration of carbohydrate material there is a

corresponding liberation of available nitrogen, as NIGHTINGALE (3) has found in the tomato. If carbohydrate respiration is a liberator of nitrogen forms which make for increased growth then carbohydrate accumulation must have previously been a binder of those nitrogen forms. Does carbohydrate accumulation inhibit vegetative extension? Do accumulating reserves check growth as well as accumulate after growth in length is checked? That could be an interpretation of the reduced growth of girdled trees. Girdling so changes other factors that this evidence might be weak. The relation of amount of elongation to composition in the shade-and-girdling series does, however, appear suggestive. The probable, if not apparent, relation of carbohydrate accumulation to reduced growth has many practical applications in connection with such questions as dwarfness, period of growth, "old age" in trees, partial etiolation, the rest period, as well as with blossom bud formation.

The "limiting factors" affecting growth may be within as well as without the plant. Clearly, relations rather than merely amounts of materials influence if they do not control vegetative response. The direct causes of growth responses should apparently be sought within the plant. At least, a fixed environment does not give a fixed response with plants of a different previous history. With agricultural perennials in a variable soil and climatic environment growth character should, then, be the basis for cultural treatment.

Summary

1. No separate environment had a consistent effect upon the composition, growth character or fruitfulness of young apple trees. For example, girdling checked growth and induced fruiting; shading of girdled trees neutralized the effects of girdling giving a growth situation comparable to ungirdled sun trees. Again, short-day trees without nitrogen grew and blossomed like long-day trees with nitrogen.

2. Growth character, including blossom bud formation, is primarily dependent upon internal composition and secondarily upon external environment. That is, of the five environmental conditions employed—sun, shade, girdling, photoperiod, nitrogen nutrient—none produced a specific growth situation.

3. The type of growth should be the condition used as a basis for deciding upon cultural practices.

4. Chemical analyses as developed at present provide only a limited and not too accurate method of measuring plant performance.

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EXPLANATION OF PLATES

Plate II. Figs. 2–5. Upper epidermis of sun, shade, sun and girdled, and shade and girdled leaves. Camera diagrams by Marian Deats Abegg.

Figs. 6–9. Lower epidermis of sun, shade, sun and girdled, and shade and girdled leaves. Camera diagrams.

Figs. 10, 11. Camera diagrams of cross-section of leaves of sun and shade leaves.

Plate III. Figs. 12–15. Camera diagrams of cross-section in pith of new wood: sun, shade, sun and girdled, and shade and girdled.

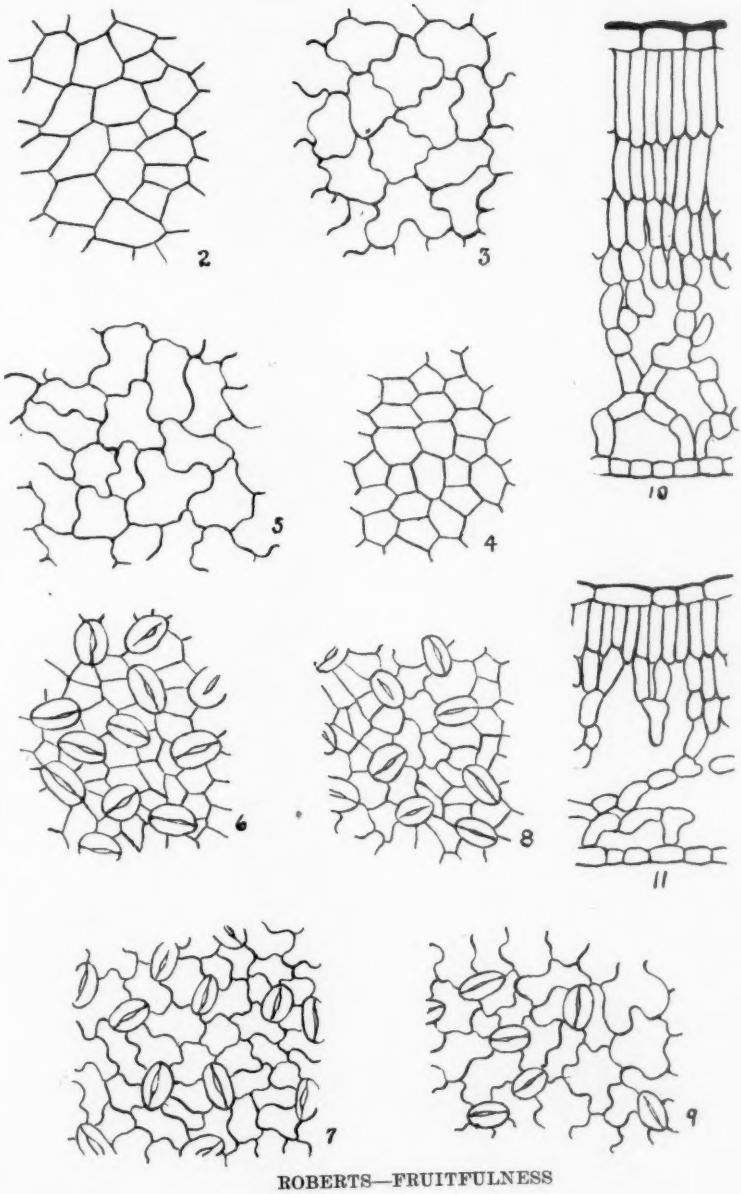
Figs. 16, 17. Pith cross-section in lower stems of sun and girdled and sun trees. Note utilization of starch and wall thickening reserves in 16.

Plate IV. Figs. 18, 19. Cross-section diagram in new growth wood of shaded and sun and girdled trees.

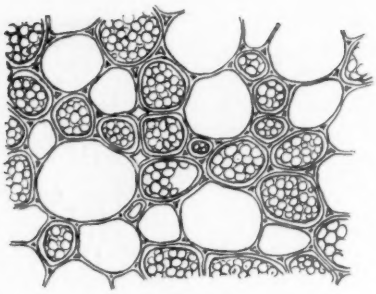
Fig. 20. Trees with nitrogen. Left, long day; right, short day.

Plate V. Fig. 21. Trees without nitrogen. Center, long day, two years; left, short day in 1925 and long in 1926; right, short day, two years.

Fig. 22. Trees with nitrogen. Right, short day in 1925, long day, 1926; left, short day, two years. Note the seasonal change in foliage character at left.



ROBERTS—FRUITFULNESS



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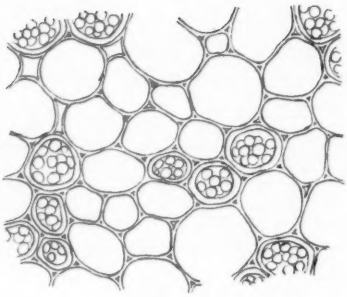

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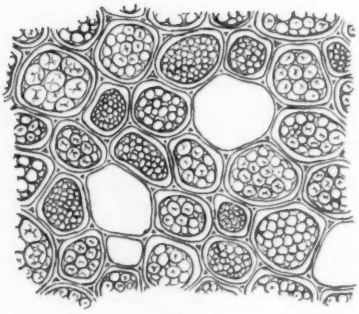

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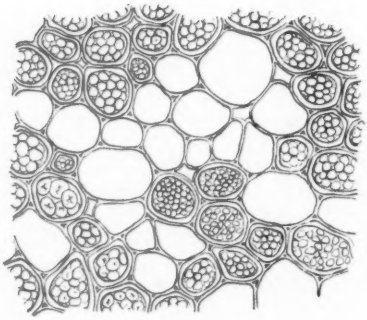

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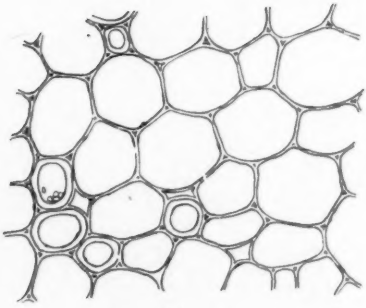

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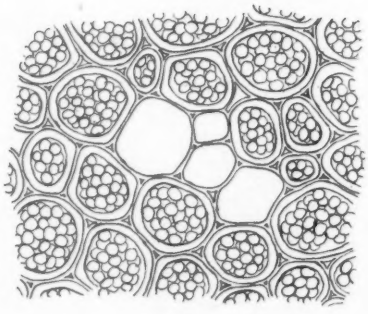

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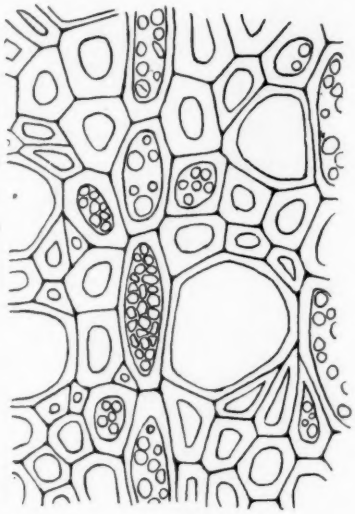

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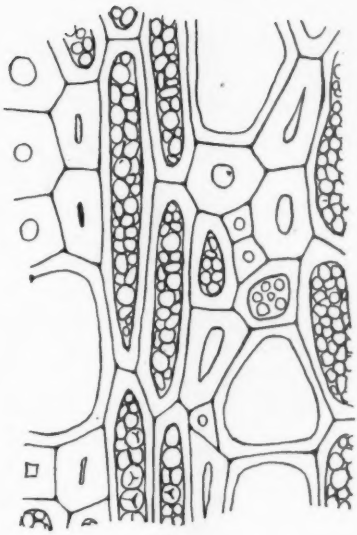

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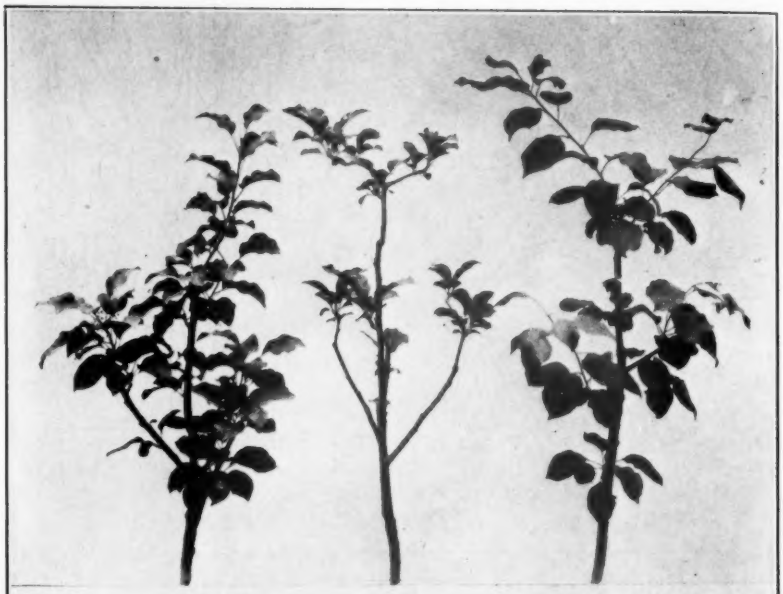
ROBERTS—FRUITFULNESS



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ROBERTS—FRUITFULNESS

TEMPERATURE AND OTHER FACTORS AFFECTING THE REST PERIOD OF POTATO TUBERS[1]

W. E. Loomis

(WITH FOUR FIGURES)

The rest period and means of breaking the rest period of seeds and stems are of very general scientific interest to plant physiologists. Some means of breaking the rest period of the irish potato is also of economic importance in those sections of the country where late varieties cannot be grown successfully, and where the high summer temperature limits the common storage period of the early crop to about three months. The fall crop of these early varieties supplies the best keeping potatoes obtainable in the South, but northern grown seed cannot conveniently be held until the late July or early August planting date, and seed from the spring crop does not germinate with usual treatments in time to produce a second crop the same season. The result is that the South either has to import 50 million bushels of potatoes or resort to less satisfactory substitutes for this important food product. At 1926–27 prices, one-fifth of the cotton produced in the older southern states would be used in exchange for potatoes if the average consumption of the country were maintained in this section.

Methods used to shorten the rest period of potatoes

A number of workers have employed chemicals of various kinds to break or shorten the rest period of potato tubers. Of these McCallum (**6**) recommended ethyl bromide; Appleman (**2**) used hydrogen peroxide; Rosa (**9**) employed nitrate of soda and later (**10**) ethylene gas; Denny (**5**), in a large number of trials, found the ethylene derivatives, ethylene chlorohydrin and ethylene dichloride, to be very effective in breaking the rest period of irish potatoes. With the exception of the nitrate of soda treatment, which is not always effective, all of these methods require special chemicals and equipment, and a skill in manipulation which is not always available in commercial practice.

Among the workers who have resorted to nonchemical means of shortening the rest period, Müller-Thurgau (**7**) was able to force potatoes in one

[1] Published with the consent of the Director as paper no. 52 of the Journal series of the University of Arkansas.

month by storing them at 0° C. In a later paper (**8**) he reported negative results with a "warmbath" treatment at 35° C. for one hour. APPLEMAN (**2**) found that treatments which facilitated the exchange of oxygen between the tuber and the atmosphere were highly effective in breaking the rest period of freshly dug tubers. In order of effectiveness he recommended peeling, covering of immature tubers so that a subdued light reached them, and cutting of mature or immature potatoes. The effectiveness of the treatments was reduced by allowing the injured surfaces to dry and by covering the treated tubers with soil instead of moss or excelsior. For these reasons his methods are not easily applied commercially.

STEWART (**12**) describes six practices as in use in various sections of the South. 1. The cull tubers are thrown back into the row at digging time and re-covered immediately. 2. The culls are placed in a shallow trench and covered with moist earth until planting time, usually about one month later. 3. The tubers are spread out in diffused light and allowed to green. 4. The tubers are spread as in three but are covered with straw and kept moist. 5. The tubers are placed in cold storage at 32° F. for three or four weeks and allowed to warm up before planting. 6. Pieces of skin are clipped from the small tubers which are to be planted whole. This practice may be combined with any of the others. CORBETT (**4**) favored a preliminary dry storage plus two weeks of treatment no. 4.

Experimental work

The work reported here considers the more practical aspects of a study of irish potato dormancy which was begun at the University of Arkansas in 1925, and which we hope to continue to a more satisfactory explanation of the phenomena involved. Both the tables and discussion are given as briefly as possible and as a result some details of manipulation are omitted. While no attempt was made in most of the experiments to secure exact control of growing temperature, moisture, etc., every precaution was taken to obtain comparable conditions by arrangement of flats, repetitions, mixing of soil, careful watering by the author, selection of equally sized tubers, etc. In experiments involving treatments given at or after planting time, pieces from the same tubers were used in each of the comparisons whenever this was possible. Unless otherwise specified, sound, disease-free tubers weighing between 3.75 and 4.25 ounces were quartered longitudinally and not more than one piece from a tuber used in a given treatment. Thus in the experiments using 40 pieces per lot, 40 tubers are represented.

The data given may conveninently be considered under three groupings: (1) Factors which affect the degree of dormancy at the time the potatoes are dug, or seed factors. (2) Factors which influence the rest period during

the interval before the crop is replanted, or storage factors, and (3) Factors which influence the germination of the planted potatoes, or soil factors.

Seed factors affecting dormancy

Variety.—It is a common observation that some varieties exhibit a more profound dormancy than others. Of the two varieties used in this work, Bliss Triumph responded more readily to both storage and chemical treatments than Irish Cobbler. No extensive variety tests were made but the author suggests, in the light of other experiments and analyses, that those varieties classed as mealy because of their normally high starch content may show deeper dormany than the varieties having a lower starch and higher protein content. It may be noted in this connection that potatoes grown under high moisture and temperature conditions frequently exhibit so little dormancy as to sprout before maturity and while the parent vine is still green. Bushnell (**3**) has shown that there is a deficiency of carbohydrates in potato plants grown under these conditions.

Fertilization.—The influence of the fertilizers applied to the growing crop upon the dormancy of the tubers produced was observed briefly. The data in table I indicate that there was a slight correlation between fertilization and the germination of the tubers produced. Unfortunately the experiment does not distinguish between the effects of nitrogen and potash and so can be only suggestive for future work in which the relation of nitrogen to the accumulation of carbohydrates, and of the K-Ca balance to permeability will be differentiated.

TABLE I

Effect of previous fertilization upon the dormancy of potatoes

Seed treatment and previous fertilizer application	Pieces sprouted	Sprouts up	Total weight sprouts
	Per cent.	Per cent.	gm.
Seed treated with ethylene			
No fertilizer	97.5	42.5	61.3
1200 pounds 4–8–4 fertilizer	100.0	75.0	61.2
1200 pounds 4–0–4 fertilizer	100.0	87.5	70.9
Seed untreated			
No fertilizer	67.5	67.5	6.5
1200 pounds 4–8–4 fertilizer	97.5	67.5	7.0
1200 pounds 4–0–4 fertilizer	92.5	75.0	9.5

Size of tuber.—Extended observations indicated that the larger tubers in a given lot of potatoes have a shorter rest period and respond more readily

TABLE II

RELATION OF TUBER SIZE TO RATE OF GERMINATION OF DORMANT POTATOES

DATE OF PLANTING	VARIETY	PREVIOUS TREATMENT	ONE OUNCE PIECES CUT FROM 4 OZ. TUBERS		CLIPPED, ONE OUNCE TUBERS	
			GERMINATION	TOTAL WEIGHT SPROUTS	GERMINATION	TOTAL WEIGHT SPROUTS
			Per cent.	gm.	Per cent.	gm.
October 21, 1925	Triumph	None	28.0	7.3	0.0	0.0
December 2, 1925	Triumph	18° storage	8.0	0.4	0.0	0.0
December 2, 1925	Cobbler	18° storage	13.3	7.7	0.0	0.0
December 2, 1925	Triumph	1° storage	84.0	87.6	76.7	81.7
December 2, 1925	Cobbler	1° storage	10.0	51.5	20.0	22.0
December 2, 1925	Triumph	28° storage	94.0	147.9	56.6	50.5
December 2, 1925	Cobbler	28° storage	73.3	41.7	56.6	6.3
November 9, 1926	Triumph	None	80.0	11.0	20.0	1.5
November 9, 1926	Triumph	None	95.0	7.9	35.0	2.6
November 9, 1926	Triumph	0.8% ethylene	70.0	239.0	17.5	21.1
December 23, 1926	Triumph	20° storage	100.0	78.8	90.0	18.8
December 23, 1926	Triumph	20° storage	100.0	75.2	85.0	24.3
Average			63.0	63.8	38.1	19.1

to various treatments than do those of smaller size. The difference appears to be sufficiently large and consistent to justify the recommendation that only tubers weighing three ounces or more be used for second crop seed where rapid germination is desired. The smaller sizes germinate as readily as the larger when both are held in storage for several months before planting, and the difference seems to be one of development or maturity.

In the experiments reported in table II, one ounce seed pieces were cut from four ounce tubers by longitudinal sectioning, and one ounce tubers were partially peeled to obtain a comparable wound stimulus. In the two series of experiments reported, the first planting date was immediately after harvest. The second planting date shows the duration of the storage condition indicated in the third column. Fifty tubers are represented in each lot in the 1925 experiments and 20 in those run in 1926. A 65 per cent. better germination and 100 per cent. greater growth per germinating sprout is shown for pieces of the same size cut from the larger tubers. Two ounce tubers gave intermediate results. The 1925 plantings were harvested on Feb. 11, 1926, and the 1926 plantings on Feb. 21, 1927.

Storage factors affecting the dormancy of potatoes

Storage treatment for shortening the rest period of potatoes, particularly if they are such as can be given on the average farm, are of especial interest from the practical viewpoint. Appleman (**2**) has stressed suberization as a factor contributing to dormancy and he found that the suberization of immature tubers could be retarded or prevented by placing them immediately in moist excelsior. Müller-Thurgau (**7**) was able to break the rest period of irish potatoes by storage at 0° C. The two factors, humidity and temperature, thus seem to be involved, and an extensive study was made of their separate and combined effects.

Humidity.—Nineteen comparisons were made in which various lots of potatoes were stored at various temperatures in air and damp moss. The temperature of the potatoes stored in moss was normally 2–4° below that of the chamber and this temperature difference probably accounts for some of the differences in growth response which were observed. When the storage temperature was moderate the dry potatoes gave the best germination; when it was high those stored in moss showed the least injury and made the best growth. The beneficial effect of the moss lay apparently in the conservation of moisture rather than in the reduction of suberization, and is in all probability related to the slow formation of wound periderm which Shapovalov and Edson (**11**) observed in shriveled tubers.

Temperature.—In the first experiments on storage temperature it was assumed that storage at 0° or 5° C. would give the best results and higher

temperatures were used only as checks. One lot of tubers was accidentally left over a steam radiator and was badly shriveled at the end of ten days. When planted, this lot surprisingly gave the best germination of any of the storage treatments used and was also superior to any of the lots treated with various salt solutions, including nitrates and sulphates. Continued experiments involving a hundred and eighty lots of potatoes have very clearly shown a shortening of the rest period by storage temperatures around 30° C. The optimum storage temperature varies with the size and the condition of the tubers used and the duration of the experiment, but a temperature of 30°–33° C. for a period of four weeks has given generally satisfactory results with 4 ounce tubers. Similar effects were obtained whether the tubers were stored in an incubator at a constant temperature or in the attic of a low roofed building having an average temperature of approximately the same degree, so that the commercial application of the method during the southern summer is a simple matter.

The experiments on storage temperature were run in three series, employing the fall crop of 1925 and the summer and fall crops of 1926. In 1925 Triumph and Cobbler, second crop tubers were harvested on October 20 immediately after the first killing frost. The check lots were planted and the storage treatments started on the following day. The checks were thus planted on October 21, the three weeks' storage lots on November 11, and the six weeks' storage lots on December 2. All treatments were harvested together on February 11 so that the growing periods for the three plantings were 112, 92, and 71 days respectively.

The potatoes stored at 16°–20° were packed in damp moss, while the others were in dry storage. The treatments marked 20°–35° were intended to have been 20°–23° and the high temperature during the second week was accidental. In addition to the irregularity of the temperature on these lots, some of them were injured by excessive temperatures.

Because of the preliminary nature of the work complete data are not given, but an average of the check and six weeks' storage treatments is included in table III. Five lots of forty pieces are averaged for each figure. The table indicates the conclusion which was drawn from the series, namely, that moist storage at moderate temperatures was no more effective than immediate planting in breaking the rest period and that, in spite of the temperature irregularities and injury to some tubers, dry storage was slightly more effective than cold storage at 0°–2° C.

In the second series, potatoes dug July 1, 1926, were held at 20° C. until July 6–8, when the check plantings were made and storage lots selected. Four storage conditions were used for four weeks' storage periods, namely, commercial storage at 0°–2° C., cellar storage at 20°–21° C., an insulated room at 23°–28° C. and an attic room beneath a low roof which had a tem-

TABLE III

EFFECT OF STORAGE TEMPERATURE UPON THE DORMANCY OF IRISH POTATOES

SERIES I

STORAGE TREATMENT FROM OCT. 21 TO DEC. 2	SPROUTS THROUGH ON FEB. 11	PIECES GERMINATED	AVERAGE TOTAL WEIGHT SPROUTS FROM 50 PIECES
	Per cent.	Per cent.	gm.
None (planted Oct. 21)	0.5	7.3	1.5
Damp moss at 18° C.	0.7	4.3	1.0
Cold storage at 1° C.	22.2	55.7	62.7
Heated room at 20°–35° C.	17.1	72.1	57.3

perature of 25°–35° C. Average temperatures for the two rooms were roughly 25° and 30°, while the cellar temperature was very constant at 20° C. Tubers stored for four weeks at the four temperatures were planted August 4 in deep flats filled with a sandy soil, and duplicate lots held at 20° and at approximately 25° to observe the germination rate. Forty one-ounce pieces, representing forty 4-ounce tubers, were used in each lot. The remaining pieces from these tubers were treated with 2 per cent. ethylene chlorohydrin by a method very kindly furnished to the author by Dr. F. E. DENNY, of Boyce Thompson Institute, and were placed with the checks at the two germination temperatures.

TABLE IV

EFFECT OF STORAGE TEMPERATURE UPON THE DORMANCY OF IRISH POTATOES

SERIES II

STORAGE TREATMENT FROM JULY 7 TO AUGUST 4	GERMINATION TEMPERATURE FROM AUG. 4 TO AUG. 31	SPROUTS UP ON AUG. 23	PIECES GERMINATED ON AUG. 31	TOTAL WEIGHT SPROUTS FROM 40 PIECES AUG. 31
		Per cent.	Per cent.	gm.
None (planted July 7)	20° C.	0.0	5.0	0.2
Cold storage—1° C.	20° C.	25.0	82.5	4.3
Cellar—20° C.	20° C.	16.3	90.0	4.6
Room—25 ± 3° C.	20° C.	43.8	85.0	8.3
Attic—30 ± 5° C.	20° C.	68.8	88.8	13.8
None (planted July 7)	25° C.	.....	87.5	7.0
Cold storage—1° C.	25° C.	37.5	52.5	5.5
Cellar—20° C.	25° C.	60.0	97.5	9.8
Room—25 ± 3° C.	25° C.	66.3	95.0	12.8
Attic—30 ± 5° C.	25° C.	100.0	100.0	16.0

A comparison of the growth made by the tubers stored for four weeks at 30° C. with the growth made by those treated with 2 per cent. ethylene chlorohydrin is given in table V. The ethylene treatment was most effective on the lots from the lower temperatures and had very little influence on the tubers from 30° storage, particularly at the higher germinating temperature, thus indicating that these lots had been brought through the rest period in four weeks. Tubers treated with ethylene on July 7 made a much better growth than the check as is shown in tables I and IX, but it is normally desirable to hold Triumph potatoes from digging time in June until the latter part of July or first of August in order to obtain the maximum benefit from the cool fall weather, so that the potatoes are actually stored for 4 to 6 weeks and the high temperature storage treatment is easily added.

Bliss Triumph potatoes planted August 1 and dug November 6 were used for the third series which was started November 8, 1926. Carefully selected lots of twenty or more tubers were stored in a series of incubators. For the lower temperatures the incubators were set up in a cold storage room. As originally planned the series was arranged in 5° intervals from 0° to 35° C., but the failure of the thermostat in the 10° chamber and injury from a mercury disinfectant used on the 25° and 30° lots reduced the comparisons finally used to those given in table VI, and illustrated in figures 1 and 2.

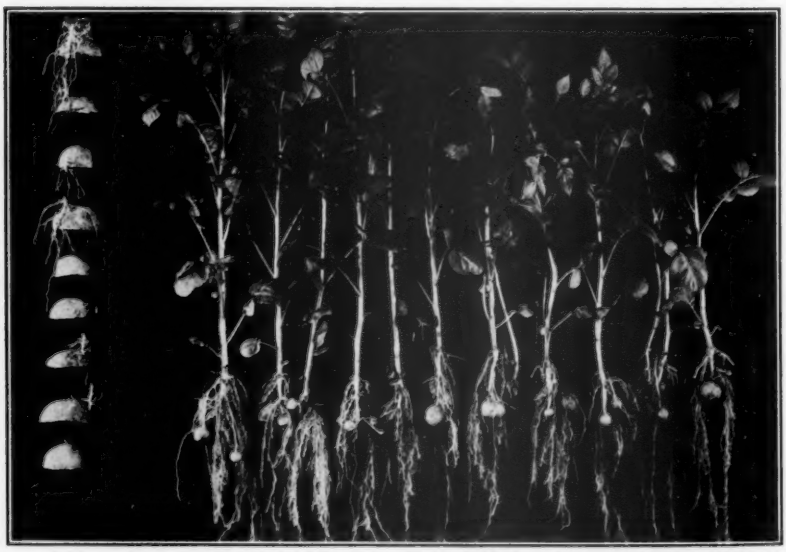

FIG. 1. Effect of storage at 35° C. upon the dormancy of potatoes. Left, planted November 8, 1926, and photographed February 21, 1927, 105 days after planting; right, stored November 8 to December 23 at 35° C. and photographed 60 days after planting.

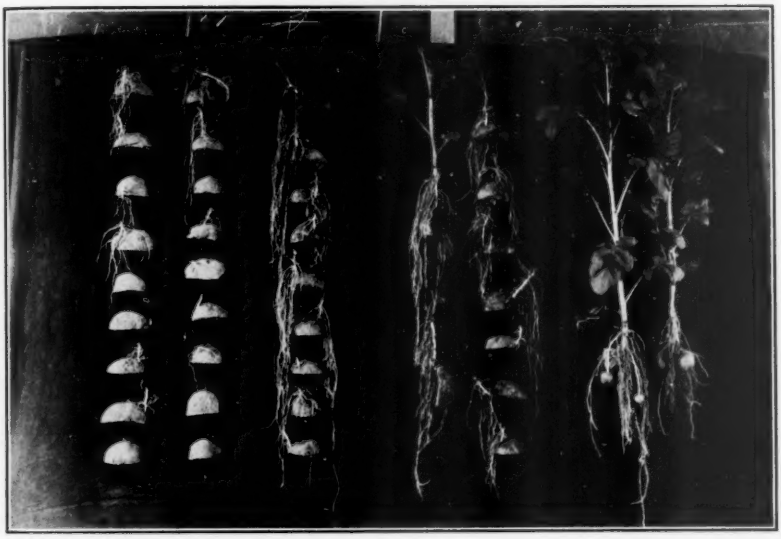

FIG. 2. Increased growth with increased storage temperature. Left to right in vertical arrangement, check planted November 8, 1926; stored at 5° C. until December 23; stored at 15° C.; stored at 20° C.; and representative plants from the tubers stored at 35° C. Photographed on February 21, 1927.

TABLE V

COMPARISON OF ETHYLENE CHLOROHYDRIN AND HIGH STORAGE TEMPERATURES FOR FORCING DORMANT POTATOES

TREATMENT	GERMINATED AT 20° C.		GERMINATED AT 25° C.	
	GERMINATION	WEIGHT SPROUTS PER 40 PIECES	GERMINATION	WEIGHT SPROUTS PER 40 PIECES
	Per cent.	gm.	Per cent.	gm.
30° storage	88.8	13.8	100.0	16.0
30° + ethylene	100.0	18.5	96.2	17.0
20° storage	90.0	4.6	97.5	9.8
20° + ethylene	95.0	19.8	91.2	18.0
Average all ethylene treatments	98.5	18.9	95.0	18.9

SOIL FACTORS AFFECTING THE DORMANCY OF PLANTED POTATOES

MOISTURE.—Moisture may be a limiting factor in the germination of second crop potatoes, particularly when they are planted on the land occupied

TABLE VI

EFFECT OF STORAGE TEMPERATURES UPON THE DORMANCY OF IRISH POTATOES

SERIES III

STORAGE TREATMENT FROM NOV. 8 TO DEC. 23	RECORD FROM 40 PIECES ON FEB. 21			
	SPROUTS THROUGH	PIECES GERMINATED	TOTAL GREEN WEIGHT TOPS	TOTAL WEIGHT NEW TUBERS
	Per cent.	Per cent.	gm.	gm.
Check (planted Nov. 8)	17.5	87.5	18.8	0.0
Incubator at 5° C.	27.5	82.5	41.8	3.8
Incubator at 15° C.	50.0	100.0	55.2	0.0
Incubator at 20° C.	85.0	100.0	154.0	21.6
Incubator at 35° C.	77.5*	77.5*	855.2	252.1

* Six weeks' storage at 35° was more than was necessary for the 4 ounce tubers and they showed injury which resulted in rot after the pieces were planted. Small tubers stored at this temperature were less shriveled and gave perfect germination but a smaller total growth.

by the first crop. Field observations in 1926 suggested that a secondary dormancy might be induced in chemically treated seed by planting in soil whose moisture content is too low for immediate germination. Under most conditions, however, moisture is more likely to be a limiting factor in yield per hill than in germination. Thus in the tests referred to above, second crop potatoes planted after fallow came up only to a 26 per cent. better stand than those planted after early potatoes; but they produced a 50 per cent. better yield per hill and an 84 per cent. better yield per acre than the potatoes on the double cropped land.

FERTILIZER.—The stimulating effect of nitrates and certain other salts, particularly sulphates, upon the germination of dormant irish potatoes suggested that row fertilization might be a factor in the germination of second crop potatoes under field conditions. A large number of experiments were run in which cut, partially dormant tubers were soaked in salt solutions of varying strengths. Sodium nitrate, sodium and potassium sulphate but especially ammonium sulphate gave a better growth than the checks. In pot and field experiments potassium sulphate gave a more rapid germination than potassium chloride, and ammonium sulphate than nitrate of soda or dried blood. Attempts to force freshly dug tubers with ammonium sulphate used as a fertilizer were less successful than the soaking experiments because of the toxic effect of the heavier applications. The toxic effect of the sulphate was, however, less than that of nitrate and it would seem to be the more desirable source of nitrogen for second crop potatoes.

In some of the early experiments, sand was compared with soil as a germinating medium for dormant tubers on the assumption that the greater aeration in the coarse sand would be advantageous. The pieces planted in soil, however, germinated more rapidly and made a greater growth than those in sand. This growth was further increased by soaking the seed pieces in M/10 nitrate solution or by adding composted manure to the soil, so that the increase would appear to be due to nitrogen fertilization. The data in table VII indicate that an increased supply of nutrients increased both sprout and root growth, but the latter more rapidly than the former. The seed used was northern grown Triumph dug about October 1 and planted December 16 when they were still dormant but largely out of the rest period. Records were taken on January 17 when the sprouts from the pieces planted in soil were just coming through. Tubers from the same lot were used in a second experiment in March after they had begun to sprout, with the result that the pieces planted in a poor, sandy soil made the best growth, followed in order by those fertilized with ammonium sulphate, acid phosphate and potassium sulphate, manure compost, and nitrate of soda. In field experiments also, row or heavy broadcast fertilization has commonly delayed the germination of potatoes fully out of the rest period.

TABLE VII

GERMINATION OF PARTIALLY DORMANT TUBERS IN SAND, SOIL, AND A SOIL AND MANURE COMPOST

SEED TREATMENT	GROWING MEDIUM	TOTAL WEIGHT FROM 40 PIECES		RATIO OF ROOTS TO TOPS
		SPROUTS	ROOTS	
		gm.	gm.	Per cent.
None	Sand	61.3	26.1	42.6
Soaked in M/10 nitrate	Sand	69.5	41.2	59.3
None	Soil	79.1	35.9	45.4
Soaked in M/10 nitrate	Soil	96.1	57.4	59.7
None	Compost	184.9	124.5	67.3
Soaked in M/10 nitrate	Compost	192.6	123.3	64.0

In another experiment, second crop tubers dug November 6 were divided into three lots, one of which was planted immediately in deep flats filled with sand and with composted soil while the other two were stored until December 23 at 20° and 35° C. They were then planted in sand and in compost until January 25. The greater growth of the stored lots when planted in compost is shown in figure 3, and the complete data are given in table VIII. Apparently the nutrients furnished by the compost stimulated the germination of the dormant tubers and increased the growth of those partially dormant.

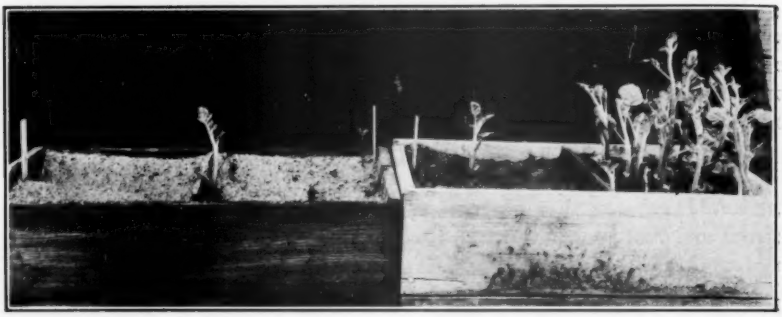

FIG. 3. Soil and temperature effects on the germination of dormant potatoes. Flat at left filled with sand and at right with soil and manure compost. Tubers in left half of each flat stored at 20° C. for six weeks before planting and those in right half at 35° C. Photographed 35 days after planting.

TABLE VIII

GERMINATION OF DORMANT AND PARTIALLY DORMANT TUBERS IN SAND AND COMPOST

STORAGE TREATMENT NOV. 9 TO DEC. 23	GROWING MEDIUM	SPROUTS UP ON JAN. 17	PIECES GERMINATED	TOTAL WEIGHT SPROUTS FROM 25 PIECES
		Per cent.	Per cent.	gm.
None (planted Nov. 9)	Sand	0.0	0.0	0.0
None (planted Nov. 9)	Compost	25.0	52.5	83.6
Incubator at 20° C.	Sand	0.0	24.0	1.9
Incubator at 20° C.	Compost	16.0	52.0	13.1
Incubator at 30° C.	Sand	76.0	100.0	72.1
Incubator at 30° C.	Compost	88.0	100.0	199.7

SOIL TEMPERATURE.—If high storage temperatures shorten the rest period, it should follow that high soil temperatures would favor the continuation of the same processes and be equally beneficial in starting the growth of dormant potato tubers. Such has been found to be the case. The average growth of the treatments in table IV which were germinated at 25° C. is 60 per cent. greater than the average of those held at 20° C., while the growth of the 25° check is several hundred per cent. better than that of the 20° check. The data in tables IX and X indicate that the effect of high soil temperatures applies to freshly dug tubers treated with ethylene chlorohydrin, and that it may be more important than the storage temperature, with untreated lots (see figure 4). Potatoes dug July 1, 1926, were planted, with and without ethylene treatment on July 7 and placed under the pre-

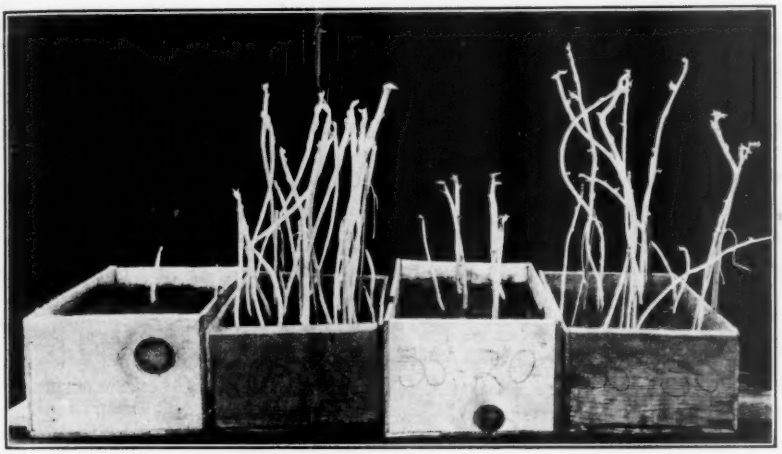

FIG. 4. Effect of storage and germination temperatures upon dormant potatoes. (Left to right, stored 6 weeks at 20° C. and germinated 4 weeks at the same temperature; stored at 20° and germinated at 32°; stored at 35° and germinated at 20°; stored at 35° and germinated at the same temperature.

viously described conditions in a cellar insulated room, and low-roofed attic. The treated lots were harvested on July 26, and the checks on August 31. Thermograph records showed that the cellar temperature was very constant at 20° C., that the temperature of the room fluctuated around 25° C., lowering somewhat toward the end of the period, and that the attic temperature was roughly 30° C. for July and 25° C. for August.

TABLE IX

RELATION OF SOIL TEMPERATURE TO THE GERMINATION AND GROWTH OF DORMANT AND TREATED POTATO TUBERS

TREATMENT	APPROXIMATE GROWING TEMPERATURE	SPROUTS UP AT HARVEST	PIECES GERMINATED	TOTAL GREEN WEIGHT SPROUTS FROM 40 PIECES
		Per cent.	Per cent.	gm.
None	20° C.	2.5	5.0	0.2
None	24° C.	40.0	80.0	4.5
None	28° C.	60.0	87.5	7.0
2 per cent. ethylene	20° C.	12.5	60.0	8.5
2 per cent. ethylene	25° C.	70.0	77.5	44.9
2 per cent. ethylene	30° C.	82.5	92.5	82.1

A second experiment was run in the fall of 1926 in which tubers stored in incubators at 20° C. and 35° C. for six weeks were replaced in incubators at 20° C. and 32° C. after planting, both storage temperatures being represented at each germination temperature. Six weeks' storage at 35° C. was excessive for this material and rot loss greatly reduced the showing of the tubers from the high temperature storage. Those pieces which germinated were, however, ahead of the pieces previously stored at 20° C. and made a 24 per cent. greater growth per growing sprout. The final growth on this experiment is shown in figure 4, while germination and growth data are given in table X.

TABLE X

RELATION OF SOIL TEMPERATURE TO THE GERMINATION OF PARTIALLY DORMANT POTATO TUBERS

STORAGE TEMPERATURE	GERMINATION TEMPERATURE	ROTTED	GERMINATED	TOTAL WEIGHT SPROUTS FROM 25 PIECES
		Per cent.	Per cent.	gm.
20° C.	20°	0.0	60.0	9.1
35° C.	20°	68.0	32.0	38.4
20° C.	32°	8.0	88.0	165.4
35° C.	32°	60.0	40.0	92.8

A comparison of the growth at 20° and 25° of potatoes treated immediately after digging (table IX) and potatoes treated a month after digging (table V) suggests that the effect of the high temperature was to complete the breaking of the rest period. Tubers treated immediately after digging made a four hundred per cent. better growth at the higher germinating temperature while there was no difference in the lots treated with ethylene four weeks after digging.

Discussion

The work reported in this paper harmonizes with previous publications on the subject of dormancy in that the successful treatments have been those which increase respiration. The observations on the possible stimulating effects of nitrogenous compounds afford an interesting basis for speculation upon the relation of starch hydrolysis and protein synthesis to dormancy and growth. It is insufficient, however, to state that we are concerned here with an application of the law of carbohydrate: nitrogen balance since the addition of nitrogen and an extended period of high respiration are conditions favoring, rather than requirements for, the breaking of the rest period of potato tubers. A study of nitrogen distribution offers more promise and our preliminary chemical studies have shown important shifts

in the colloidal and non-colloidal nitrogenous fractions. Such changes are, however, more probably the result of sugar accumulations from hydrated starch than the cause of this reaction. APPLEMAN (**1, 2**) was unable to correlate the rest period with enzyme changes, although he found sugar accumulation characteristic of changes which took place toward the end of the rest period when the tubers were stored at low temperatures. It would seem that dormancy is more probably related to cytoplasmic structure than to chemical composition or enzyme formation and that the rest period is broken by some such change as the reversal of lipoidal, proteinaceous phases in the cytoplasm whereby the permeability of the cell is increased and the enzymes associated with the protein phase are liberated. Such a theory is in harmony with the efficiency of fat solvents such as ether and ethylene in shortening the rest period and with the more rapid rate of water loss from tubers which are out of dormancy, but is not easily demonstrated experimentally.

Summary

Experiments are reported in which the rest period of dormant irish potato tubers was broken in four weeks by storage during July in the attic of a low-roofed building. Extended studies have established the efficacy of storage temperatures around 30° C. in shortening the dormant period of potato tubers and have demonstrated the importance of high soil temperatures for the rapid germination of partially dormant tubers.

Three and four ounce tubers selected from a given lot of freshly dug potatoes have germinated more readily and made a stronger growth than one and two ounce tubers from the same lot. The difference is lost as the tubers pass through the rest period.

Composted soils and low concentrations of nitrogenous fertilizers had a stimulating effect on the germination of dormant and partially dormant tubers which was not observed in tubers which were completely out of the rest period.

Storage in damp moss, as compared to dry storage, at high temperatures has reduced shriveling and rot loss subsequent to planting, presumably because of the more rapid formation of a wound periderm in the turgid tubers, but has had no direct effect upon the rest period.

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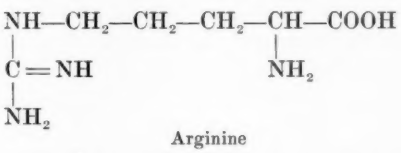
THE BASIC NITROGEN OF PLANT EXTRACTS*

HUBERT BRADFORD VICKERY[1]

The estimation of the proportion of the nitrogen of a plant extract which occurs in the form of simpler nitrogenous bases is a problem of great importance. Although the physiological significance of many of these substances can, at present, only be guessed at, there is no doubt that they play an important part in metabolism, and if adequate information could be obtained it is probable that many relationships which are now obscure might become clear.

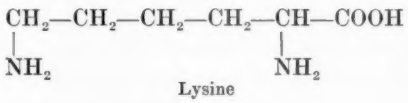
It is useful to distinguish those of the simpler bases which bear a direct relationship to the amino-acid constituents of proteins from other bases which are found in plants. Arginine (I), lysine (II), and, to a lesser extent, histidine (III), are well known in extracts of plant material. Methods for

I



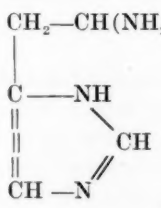
Arginine

II



Lysine

III



Histidine

the approximately quantitative isolation of these three substances from the products of hydrolysis of proteins have been developed, and the general prin-

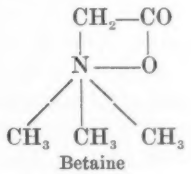
* The expenses of this investigation were shared by the Connecticut Agricultural Experiment Station and the Carnegie Institution of Washington, D. C.

[1] Prepared on invitation from the Committee on Methods of Chemical Analysis, American Society of Plant Physiologists.

ciples underlying these methods are applicable to the study of plant extracts.

The betaines (IV, V) are formally related to the protein amino-acids since they may be regarded as methylation products of these substances.

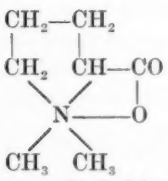
IV



Betaine

(Type substance derived from glycocoll)

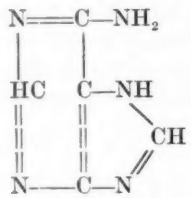
V



Stachydrine

(The betaine of proline)

VI

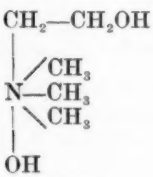


Adenine

They occur widely in plants and may form no inconsiderable part of the total basic nitrogen of the extract. Unfortunately we have no clean cut methods for dealing with the individuals of this group. To be sure there is a general precipitant, mercuric chloride in acid solution, but the separation of the individual betaines that may be found in one extract is largely a matter of fractional crystallization of suitable salts.

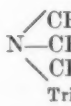
Beside those bases which may be regarded as related to the proteins, there are at least two other distinct types of substances which are usually encountered in plant extracts. These are, on the one hand, the weakly basic purines (VI) and, on the other, relatively strong bases such as choline (VII) and trimethylamine (VIII). Purines are intimately associated with

VII



Choline

VIII



Trimethylamine

(Free base analogous to ammonia, NH_3)

nucleic acid which is supposed to be present in cell nuclei, while choline is known to be associated with the phosphorized fats, and may possess other, as yet unsuspected, affiliations.

In the present discussion no account will be taken of that complex and economically important group of plant bases known as alkaloids. These do not seem to be nearly so widely distributed as the simpler bases and, moreover, alkaloid chemistry possesses a technique of its own which differs from that required in the study of the simple bases.

It is necessary to appreciate that these types of substances, which have been mentioned as probable components of any particular plant extract, by no means exhaust the possibilities. We know of their presence in plants simply because they, for the most part, possess properties which render isolation and identification easy. But there is every reason to expect that further study may reveal the presence of still other basic substances which, while they may be known as chemical possibilities or as specimens in the museum of the organic chemist, have not yet been shown to occur in nature.

The problem of the investigation of the bases which occur in plant extracts may, for the present purpose, be reduced to that of the choice of suitable reagents. This choice is severely circumscribed by two general requirements: first, the reagent must not introduce nitrogen and, second, it must be readily and completely removable by some manipulation which does not introduce conditions that might be expected to alter the other organic substances in the solution. These requirements cannot always be met; in fact it is seldom that we can remove reagents and, at the same time feel confident that other components of the solution have undergone no change. We have, therefore, a condition in which judgment and experience with each individual plant extract must play a dominant part. General prescriptions are, at the present time, of doubtful validity and may prove highly mischievous.

Phosphotungstic acid has been employed for many years as a general precipitant for bases and, when dealing with the mixture of amino-acids that is obtained from the hydrolysis of proteins, it is fairly definite in its action. With plant extracts, however, many circumstances combine to render a determination of the nitrogen precipitated by this reagent an uncertain measure of the true basic nitrogen.

To illustrate this statement, experiments[2] carried out with extracts from the alfalfa plant and from yeast may be described. The alfalfa extract was prepared by moistening the ground material (leaf and stem) with ether and pressing out in the hydraulic press. The juice was rapidly heated to 81° C.

[2] These experiments have been in part more fully described in papers in the Jour. Biol. Chem. See bibliography.

to destroy enzymes and coagulate proteins, filtered and concentrated, and alcohol was then added to make a final concentration of 50 per cent. by volume. The precipitate so produced was removed and the filtrate concentrated. This solution contained no detectable quantity of proteins, and is referred to in the following as "alfalfa filtrate." In order to remove phosphates and also those organic acids which form insoluble barium salts, an excess of barium hydroxide was added and the precipitate removed. An equal volume of alcohol was then added to the filtrate and a second precipitate removed. The first precipitate contained phosphoric and citric acids but little nitrogen, while the second contained malic and malonic acids[3] together with nitrogenous compounds accounting for nearly 14 per cent. of the total nitrogen of the protein free extract. The filtrate from these precipitates was freed from reagents and treated with mercuric acetate, sodium carbonate and alcohol (NEUBERG's reagent) (**1, 3, 4**), a procedure which precipitates amino-acids, purines, and bases containing an amino group very completely, but which does not precipitate the methylated bases. This precipitate was decomposed with hydrogen sulphide and the solution was assumed to contain the greater part of those amino-acids and bases, exclusive of betaines and other methylated bases, which occur in the juice of the alfalfa plant. In order to separate the basic substances, phosphotungstic acid was then added in the usual way, and the precipitate decomposed by grinding with barium hydroxide five times, first in the cold and finally at boiling temperature. The fifth extract appeared to contain little, if any, organic material.

The filtrate from the phosphotungstic acid precipitate was treated with an excess of barium hydroxide to remove phosphotungstic and sulphuric acids and the precipitate thoroughly washed. Finally the excess of barium hydroxide was exactly removed from both fractions by means of sulphuric acid. This is the customary procedure for the separation of bases from non-basic substances. The data which were obtained are given in table I.

In view of these results what is one to take as the indicated basic nitrogen? Is it the nitrogen of the base fraction, amounting to 19.6 per cent. of the original nitrogen of the alfalfa filtrate, or should the nitrogen which could not be removed from the barium phosphotungstate and sulphate precipitate, amounting to 4.56 per cent. more of the original nitrogen, be added?

A still more ambiguous case was encountered in an examination of an extract from yeast. The precipitate obtained with mercuric acetate and sodium carbonate was divided into two parts, one of which was soluble in 5 per cent. sulphuric acid, and the other insoluble. This part contained 33.4

[3] Unpublished data. For the presence of these hydroxy-acids in alfalfa, see citation (**2**).

TABLE I

THE DISTRIBUTION OF NITROGEN OBTAINED BY MEANS OF PHOSPHOTUNGSTIC ACID IN A FRACTION FROM ALFALFA FILTRATE

FRACTION	WEIGHT	PROPORTION OF NITROGEN OF ALFALFA FILTRATE
	gm.	Per cent.
Total nitrogen of solution	28.60	51.9
Ammonia nitrogen of solution	1.54	.....
Total nitrogen of base fraction	10.81	19.6
Ammonia nitrogen of base fraction	0.32	.....
Nitrogen in Ba phosphotungstate and $BaSO_4$ (base fraction)	2.57	4.56
Total nitrogen of filtrate from bases	12.46	22.6
Ammonia nitrogen of filtrate from bases	0.24	.....
Nitrogen in Ba phosphotungstate and $BaSO_4$ (filtrate from bases)	1.40	.....
Total nitrogen recovered	23.27	42.19
Nitrogen lost in Ba phosphotungstate and $BaSO_4$	3.97	7.1
Ammonia nitrogen lost*	0.98	.....
Nitrogen unaccounted for	0.38	.....

* This ammonia was lost from the alkaline solutions employed in the removal of phosphotungstic acid as its barium salt.

gm. of nitrogen, of which 13.84 gm. were obtained in the base fraction, while no less than 6.65 gm. were retained by the barium phosphotungstate and sulphate. In this case it was a question whether one should regard 13.84 gm. as the basic nitrogen of this fraction, or 20.49 gm., *i.e.*, 21.5, or 31.8 per cent. of the original nitrogen of the yeast extract.

A partial explanation of the difficulty was obtained by an examination of the barium phosphotungstate precipitate. It was boiled with concentrated hydrochloric acid for an hour, diluted and filtered. This extracted nearly one-quarter of the nitrogen. Further treatment with acid, finally boiling for 24 hours with 20 per cent. hydrochloric acid, extracted nearly another quarter of the nitrogen. The hot extracts on cooling deposited barium chloride, and, as they contained much free phosphotungstic acid, the small amount of organic bases likewise present separated as phosphotungstates. These were separated from the barium chloride by extraction with acetone. In all somewhat over one gram of nitrogen which appeared to belong to basic substances, and over two grams of nitrogen which appeared to belong to simple mono-amino-acids, were obtained. The basic substances were not further investigated, but the mono-amino-acid fraction yielded

appreciable quantities of glutaminic and aspartic acid while, in addition, a mono-amino mono-carboxylic acid fraction was obtained which, on evaporation, deposited crystals resembling leucine and valine. The occurrence of a positive Millon's reaction indicated the presence of tyrosine, while the fact that the amino nitrogen amounted to only 63 per cent. of the total nitrogen, in spite of the severe hydrolysis to which the solution had been subjected, indicated that proline might also be present.

To sum up, this experiment shows that, in addition to basic substances, phosphotungstic acid precipitated compounds, probably polypeptides, which yield simple mono-amino-acids on hydrolysis. It is, of course, impossible to determine from the present evidence whether the mono-amino-acids were associated with bases as basic polypeptides or whether we were dealing with polypeptides containing only mono-amino-acids. The fact remains that by no means all of the nitrogen precipitated by phosphotungstic acid belonged to strictly basic substances.

Examination of the solution of, presumably, basic substances obtained from alfalfa filtrate by decomposition of the phosphotungstates with barium hydroxide has also given evidence of the presence of nitrogen in substances which are not bases. The standard procedure with such a solution is to obtain fractions representing silver compounds insoluble in acid (the purine fraction), silver compounds insoluble in alkali (the histidine and arginine fraction), and a filtrate (the lysine fraction). Further work is necessary before these fractions can be separated from each other with certainty, and still more before we can isolate the individual substance quantitatively from each fraction. However, it may be illuminating to describe the results obtained from the lysine fraction, as they illustrate the difficulties encountered in this type of work. This solution contained four grams of nitrogen or 7.4 per cent. of the nitrogen of the alfalfa filtrate. It was treated with an excess of mercuric chloride and neutralized to litmus. The resulting precipitate contained 2.6 gm. of nitrogen. The filtrate was made alkaline and a second precipitate removed which contained 1.0 gm. of nitrogen. From the first precipitate less than 0.1 gm. of nitrogen was obtained as lysine by crystallization of the picrate. The mother liquors were freed from picric acid and a determination of total and amino nitrogen indicated that 38.9 per cent. of the nitrogen occurred as α-amino groups. The material was subjected to hydrolysis with acid after which it was found that 71 per cent. of the nitrogen occurred in α-amino groups. The solution was next treated with phosphotungstic acid, the precipitate decomposed, and nearly one-half a gram of nitrogen was then recovered as lysine picrate. The filtrate from the lysine phosphotungstate, when freed from reagents, yielded tyrosine and a mixture of leucine and valine with other amino-acids.

A similar type of material was found in the alkaline mercury precipitate. No lysine could be obtained from this solution at all until it had been subjected to hydrolysis. The hydrolysis raised the proportion of α-amino nitrogen from 51 to 81 per cent. and small amounts of lysine could then be readily obtained by crystallization of the picrate. Tyrosine and other mono-amino-acids were present in the mother liquors.

It is evident, therefore, that the "lysine fraction" obtained by the usual procedure contained much nitrogen in forms other than lysine, and it is highly probable that we were here dealing with a mixture of polypeptides in some of which lysine was a constituent.

It would be tedious to continue the description of the behavior of these different basic fractions. The purine fraction consisted essentially of purines with little extraneous material, but in the arginine fraction, as in the lysine fraction, there were peptides, and some of these may have contained arginine as a constituent.

Taken together, therefore, it seems clear that we are not yet in a position to assume that the nitrogen precipitated by phosphotungstic acid from plant extracts is wholly basic in nature, and it would not be going too far to state that this reagent is far less selective than it is sometimes thought to be. This must not be taken to mean that we should discontinue its use; it simply means that the results obtained with phosphotungstic acid must be regarded as furnishing outside limits to the true basic nitrogen. Further examination of the material precipitated by it is necessary.

In this laboratory extensive use has been made of a procedure devised by NEUBERG and KERB (**1**) for the precipitation of certain basic substances as well as amino-acids. Mercuric acetate and sodium carbonate are added alternately in such a way as to maintain an alkaline reaction until further addition of either solution produces a yellow precipitate. At this point an equal volume of alcohol is added to promote flocculation of the precipitate. To illustrate the effectiveness of this procedure as a means of precipitating amino nitrogen the results of an examination of a hot-water extract from yeast may be quoted. This extract contained 57.2 gm. of total, and 23.4 gm. of amino nitrogen. The filtrate from the mercuric acetate and sodium carbonate precipitate contained 5.2 gm. of total and only 0.67 gm. of amino nitrogen. As the volume of the filtrate measured over 26 liters, the small amount of amino nitrogen which escaped precipitation probably represents the solubility of the mercury compounds.

The advantage of this procedure is that it affords a sharp separation of bases containing methylated amino groups, such as choline and the betaines (IV, V, VII, and VIII) from other basic substances and from amino-acids. In the examination of alfalfa filtrate a solution containing 41.1 gm. of total and 10.0 gm. of amino nitrogen was treated in this way. The precipitate

contained 28.6 gm. of total and 10.1 gm. of amino nitrogen, while the filtrate contained 7.89 gm. of total and only 0.31 gm. of amino nitrogen. The slight discrepancy in the quantities of amino nitrogen recovered is probably due to experimental error, while the loss of 4.6 gm. of nitrogen is mainly due to the 3.38 gm. which could not be recovered from the large amount of mercuric sulphide obtained when decomposing the mercury precipitate. Of the 7.89 gm. of nitrogen in this filtrate, 4.67 gm. belonged to substances precipitable by phosphotungstic acid and 3.68 gm. of this nitrogen were obtained as crystalline derivatives of stachydrine (V) and choline (VIII) (**4**), while traces of betaine (IV) and trimethylamine (VIII) were also present. Taking into consideration the difficulty of isolating crystalline derivatives in quantitative yield it is apparent that this basic fraction contained little except bases of the methylated type.

A similar fraction from yeast extract consisted almost entirely of choline and nicotinic acid (**5**).

The results with these fractions are in marked contrast to those obtained when phosphotungstic acid precipitates are produced in solutions containing the amino-acids and peptides present in the original extract. The nitrogen precipitated by phosphotungstic acid from these less complex solutions appears in fact to consist entirely of basic nitrogen. The conclusion to be drawn is that it would be advisable to accept the total nitrogen precipitated by phosphotungstic acid as a measure of the basic nitrogen only when this precipitate has been produced in a relatively simple mixture. Precipitation with this reagent can be regarded, in general, only as a preliminary step.

Our present information indicates that extracts obtained from plants are usually extremely complex mixtures. Consequently their chemical examination cannot fail to be difficult and laborious. Too much account cannot, as yet, be taken of the results of indirect methods of analysis. When a substance has once been shown to be present we are, to some extent at least, justified in using a short-cut analytical method for its estimation. But in the lack of such information, the interpretation of the results of these methods becomes a matter of speculation and uncertainty. This may sound discouraging to plant physiologists but it must be remembered that we have well nigh passed through the era of simplification in science. The older beautifully simple statements of the physical laws of nature are already undergoing a process of complication. Second and third terms are being added to our equations to care for the slight discrepancies which are found when more accurate methods of measurement are employed. Animal physiology is becoming a science in which a broad grounding in physical chemistry is increasingly necessary and it is certain that plant physiology is des-

tined to become a field in which organic chemistry will play an even more significant part than it does at present.

The facts that lysine occurs in this plant, and choline in that, are interesting, but not as yet important. What is important, however, is to get a broad insight into the chemical environment in which the process of life is going on. The chemically trained physiologist and the physiologically trained chemist will then, perhaps, be able to attain to a clearer conception of what this process may be.

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NEW HAVEN.

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ELECTRODIALYSIS AS A MEANS OF STUDYING BIOCHEMICAL DIFFERENCES IN ABNORMAL APPLE TISSUE*

JAMES C. MOORE, R. G. REEVES, AND R. M. HIXON

(WITH SIX FIGURES)

Within recent years electrodialysis has come into favor as a means of studying certain chemical and physical phenomena. This method has been employed by DHÉRÉ and GORGELEWSKI (**4**), ADOLF and PAULI (**1**), FREUNDLICH and LOEB (**6**), BERNHARD and BEAVER (**2**), and ETTISCH and BECK (**5**) as a means of determining the composition of serums; by LISBONNE and VIELQUIN (**10**), and FRICKE *et al.* (**7**) in their investigations of enzyme activity. TAYLOR, BROWN, and SCOTT (**15**) used electrodialysis for separating the active and inactive constituents of insulin; HOFFMAN and GORTNER (**8**) found it a suitable means for splitting free agar acid from its calcium salt. KÖNIG (**9**), MATTSON (**11**), and CLARK *et al.* (**3**) have studied the replaceable bases of soils by the same method.

From the work of CLARK just mentioned on the electrodialysis of soils, the idea of testing this method for the study of biochemical phenomena of plant tissues was conceived, and as a review of the literature at hand revealed no report of any such investigations, the present study was undertaken.

Description of electrodialysis cell

MATTSON (**11**) has described a three-chambered cell which he used for the study of replaceable bases in soils. CLARK, HUMFELDT and ALBEN (**3**) modified the MATTSON cell by putting in a cooling system made of ordinary glass tubing. The diagrams in figs. 1 and 2 show the essential features of such a cell. A^1 and A^2 in fig. 1 are made of hard rubber, $\frac{3}{8}$ x 8 x 6 inches, with an outlet at the bottom for draining the cell. B, C, and D are made of $\frac{3}{4}$-inch soft rubber as shown in fig. 2. E^1 and E^2 are parchment paper membranes. The entire cell is clamped together by means of six bolts. The above cell warped badly due to the strain of the bolts when tightened. To overcome this and to allow for greater space for biological material, the following modifications were made: (1) the cell was made of soft rubber throughout; (2) the $\frac{3}{8}$-inch soft rubber ends were reinforced with $\frac{1}{8}$-inch

* Contribution from the Pomology Section and the Department of Chemistry, Iowa State College.

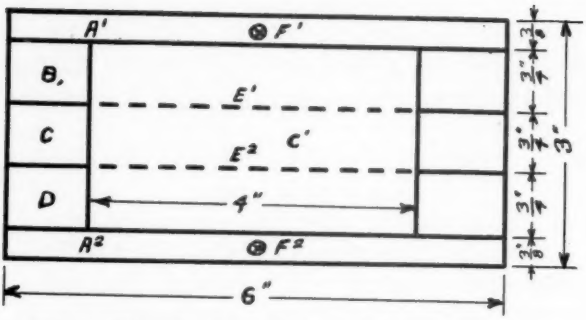

FIG. 1. Three chambered electro-dialysis cell. Top view.

brass plates; (3) the cell was made nine inches high, giving a space of $4 \times 8 \times \frac{3}{4}$ inches for the material under investigation. This compartment will readily hold 200 grams of apple parings (wet weight) and the other two compartments about 300 milliliters of water. F^1 and F^2 are ordinary binding posts for connecting the electrodes and lead wires. The electrodes, described by CLARK *et al.* (*loc. cit.*) were used. They consisted of a platinum wire gauze for the negative cell and a three wire electrode for the positive cell.

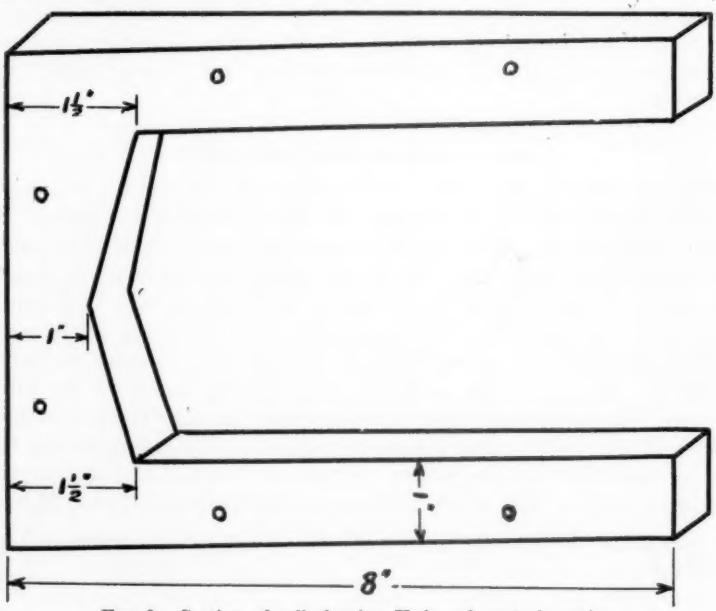

FIG. 2. Section of cell showing U-shaped central portions.

Materials and methods

The materials used for this investigation were normal and abnormal Jonathan apples.

The normal apples were free from surface blemishes, of good size and color, complying with the requirements for A grade fruits. Abnormal apples were of the same general size, color and condition of flesh, but were badly affected with a superficial dark brown or black circular spot commonly known as Jonathan spot. In most cases about 50 to 75 per cent. of the total area of the apples was affected; according to the arbitrary classification used by PLAGGE and MANEY (**13**) apples of this type would fall into the bad class.

These apples had been in storage from October, 1926, to June, 1927. They were from the same orchard and so far as known were handled in a similar manner throughout the period of storage.

The fruit was peeled with a small hand-operated apple parer, the cutting blade being adjusted to peel as thin as possible, which gave a peel about 1 to 2 millimeters in thickness. The parings were immediately weighed on a trip balance and then placed in compartment C^1 of the cell described in fig. 1. Two hundred milliliters of distilled water were then poured into compartments A and B, and the peels in C^1 were covered with distilled water.

A direct current of 110 volts was used for all samples. The amperage was recorded at the end of each time interval, which was taken from the closing of the circuit with the addition of water to the cell, to the breaking of circuit by removing the dialysate from the compartments. The time required to drain and refill these compartments varied from two to three minutes. A twenty-minute period, therefore, actually would be about seventeen to eighteen minutes. The dialysate was drained into 300 ml. Erlenmeyer flasks, which were tightly closed with rubber stoppers until they could be titrated. The material which came out on the basic side was titrated electrometrically,[2] using a student potentiometer, with 0.1 N HCl which had been standardized against $AgNO_3$.

The solution from the cathode cell was titrated with 0.1 N NaOH using phenolphthalein as an indicator. Preliminary runs were made using varying amounts of tissue and various time intervals for changing the dialysate. The final method adopted for the experiment follows: The cell was thoroughly cleaned, new parchment membranes put in place and then dialyzed for a period of one hour with frequent changes in order to remove inorganic

[2] The pigment of the Jonathan apple migrates to the negative cell; this gives a solution with sufficient color to mask an indicator. Further work is going forward on this phenomenon.

material from the paper. A weighed quantity (in each case 184.5 gm.) of wet apple parings was placed in the cell and the current turned on. The time intervals used were two twenty-minute periods, one thirty minutes, two one-hour, one two-hour, two four-hour, and one twelve-hour periods. This makes a total of twenty-five hours and ten minutes elapsed time.

These intervals for changing dialysate proved very satisfactory for keeping the temperature of the middle compartment below 55° C.; a temperature above this was considered to be injurious to the tissue. In only two cases did the temperature go above 45° C., both of these being in series "3a."

After titration the dialysates were grouped according to the initial E.M.F. and evaporated to dryness on a sand-bath and ashed for the purpose of qualitative analyses.

The series were indicated as normal and abnormal; 1 N, 2 N, 3 N, and 4 N being the four different samples of normal apple parings, and 1 A, 2 A, 3 A, and 4 A being the four different samples of abnormal tissue. As previously mentioned 1 N, 2 N, 1 A and 2 A were run in different ways and will not be used except for comparison of methods. 3 N, 4 N, 3 A and 4 A were run identically and consisted of equal quantities of tissue. Therefore, these samples are comparable.

Results

Table I gives the time interval of changing dialysate, the ml. of normal acid, and the ml. of normal base of dialysate as calculated from titration of the dialysate at the end of each period.

TABLE I

TIME OF CHANGING DIALYSATE AND MILLILITERS OF ACID OR BASIC CONSTITUENT AT END OF EACH PERIOD

1 N			2 N		
N ACID	TIME	N BASE	N ACID	TIME	N BASE
ml.		ml.	ml.		ml.
1.725	1 hour	5.511	0.334	15 minutes	1.234
0.799	1 hour	1.073	0.826	45 minutes	2.179
0.431	1 hour		0.684	1 hour	0.254
0.212	1 hour		0.317	1 hour	
0.200	1 hour		0.216	1 hour	
0.211	1 hour		0.220	1 hour	
0.183	1 hour		0.312	2 hours	
0.148	1 hour		0.275	2 hours	
0.174	1 hour		0.321	3 hours	
0.138	1 hour		0.50	4 hours	
0.128	1 hour		0.780	8 hours	
0.156	1 hour		0.50	12 hours	
2.065	12 hours 40 minutes		1.00	4 hours 5 minutes	
6.570		6.584	5.485		3.667

Table I indicates that practically all of the basic constituents were removed from 1 N during the first hour, likewise they were removed from sample 2 N, in which case the time interval was different and also a different weight of tissue. In case of sample 1 N the ml. of normal acid are practically equivalent to the basic constituents. On the other hand in 2 N the normal acid is considerably higher than normal base.

Table II gives the physical and chemical measurements of the above samples. The amperage and temperature were taken at the end of each time interval. The E.M.F. was determined by the student potentiometer. The pH was taken from tables compiled by SCHMIDT and HOAGLAND (**14**).

TABLE II

PHYSICAL AND CHEMICAL MEASUREMENTS OF SAMPLES 1 N AND 2 N

AMPERAGE		INITIAL E. M. F.		pH		TIME		TEMPERATURE	
1 N	2 N	1 N	2 N	1 N	2 N	1 N	2 N	1 N	2 N
						hours	hours	°C.	°C.
0.89	0.5	0.97	0.918	11.63	10.72	1.0	0.25	47	30
0.41	0.5	0.857	0.925	9.68	10.85	1.0	0.75	30	37
0.20	0.31	0.475	0.80	3.2	8.74	1.0	1.0	22	26
0.09	0.1	0.559	0.500	4.6	3.66	1.0	1.0	19	21
0.06	0.05	0.545	0.475	4.4	3.24	1.0	1.0	21	19
0.02	0.04	0.534	0.575	4.2	4.93	1.0	1.0	20	20
0.01	0.05	0.496	0.55	3.5	4.51	1.0	2.0	21	19
0.06	0.04	0.502	0.52	3.6	4.0	1.0	2.0	21.2	18
0.04	0.04	0.505	0.485	3.7	3.41	1.0	3.0	21	19

Table II shows that the time interval has considerable effect upon the conductivity of the solution as shown by the amperage. 2 N in the first two periods has almost one-half the amperage of 1 N, but in each of the other periods the amperage is higher. Sample 1 N weighed 163 gm. while sample 2 N weighed 123 gm.

Tables III and IV show comparisons for similar data on abnormal tissue, the wet weight of tissue being the same in each sample. However, in 1 A, the time interval was varied slightly and the electrode was the same as in the other runs, *i.e.*, platinum wire gauze and one trident-shaped three-wire platinum electrode. In 2 A both electrodes were trident.

Table III indicates that the size of the electrode has considerable influence on the rate of dialysis, as is indicated by the amperage and quanity of titratable acids and bases which were removed from the tissue. Table IV gives the physical measurements of the same samples.

The amperage of 2 A as shown in table IV goes through a gradual increase to a maximum of 0.6 at the end of two hours and ten minutes, then

TABLE III

MILLILITERS OF ACID AND BASE IN DIALYSATE AT END OF EACH PERIOD

1 A			2 A		
TIME	ACID	BASE	TIME	ACID	BASE
Minutes	ml.	ml.	Minutes	ml.	ml.
20	0.587	3.199	20	0.376	1.153
20	0.413	1.914	20	0.174	0.265
20	0.358	1.233	30	0.293	0.945
30	0.661	1.948	60	1.084	2.894
30	0.514	0.922	60	1.046	1.464
60	0.973	0.980	120	1.358	0.922
60	*	0.034	240	1.927	
120	*	0.000	240	1.239	
240	1.322	0.000	720	2.193	
240	1.001	0.000			
720	2.130				

* Sample lost.

TABLE IV

PHYSICAL MEASUREMENTS OF ABNORMAL SERIES 1 A AND 2 A

AMPERAGE		INITIAL E.M.F.		pH		TIME		TEMPERATURE	
1 A	2 A	1 A	2 A	1 A	2 A	1 A	2 A	1 A	2 A
						Minutes	Minutes	°C.	°C.
0.85	0.4	0.987	0.945	11.9	11.19	20	20	35	26
0.55	0.25	0.974	0.913	11.67	10.65	20	20	33	23
0.49	0.30	0.956	0.919	11.37	10.75	20	30	29	23
0.56	0.60	0.970	0.970	11.60	11.60	30	60	30	23
0.41	0.5	0.930	0.939	10.93	11.09	30	60	26	31
0.45	0.45	0.918	0.928	10.72	10.89	60	120	31	30
0.36	0.44	0.711	0.587	7.23	5.13	60	240	24	27
0.18	0.15	0.584	0.621	5.08	5.71	120	240	22	22
0.13	0.18	0.540	0.598	4.34	5.32	240	720	23	22
0.10	0.12	0.545		4.42		240	1020	21	23
0.20		0.615		5.6		720		21	

gradually drops to 0.12 at the end of 25 hours and 10 minutes. When a platinum gauze is used for the negative electrode the amperage begins at a maximum and drops rapidly to the end of the fourth hour, after which there is a gradual decrease.

Table V shows the milliliters of normal acid and base of the dialysate from normal apple peels at the end of each period, and the total amount removed. Both samples were identical and were treated in the same manner; therefore they serve as checks.

TABLE V

MILLILITERS OF ACID AND BASE IN 184.5 GRAMS OF NORMAL APPLE PEELS

TIME	3 N		4 N	
	ACID	BASE	ACID	BASE
Minutes	ml.	ml.	ml.	ml.
20	0.688	3.678	0.523	2.44
20	0.376	1.616	0.303	1.568
30	0.597	1.591	0.431	1.648
60	0.991	1.095	1.092	1.360
60	0.881	0.075	0.551	0.104
120	0.927		0.798	
240	1.386		0.918	
240	0.707		0.706	
720	1.377		1.450	
Total 1510	7.930	8.055	6.772	7.120

While there are variations in the above checks the difference is very insignificant as compared to the large differences between normal and abnormal tissue. Table VI shows the physical measurements of the same tissue.

TABLE VI

PHYSICAL MEASUREMENTS OF NORMAL TISSUE

AMPERAGE		INITIAL E.M.F.		pH		TIME	TEMPERATURE	
3 N	4 N	3 N	4 N	3 N	4 N	3 N AND 4 N	3 N	4 N
						Minutes	°C.	°C.
0.95	0.75	0.983	0.983	11.83	11.83	20	40	32
0.65	0.52	0.960	0.974	11.44	11.61	20	35	34
0.55	0.51	0.959	0.973	11.42	11.66	30	35	34
0.40	0.52	0.920	0.949	10.75	11.25	60	35	38
0.30	0.28	0.80	0.836	8.7	9.34	60	30	26
0.24	0.20	0.6	0.512	5.4	3.85	120	30	25
0.21	0.2	0.58	0.568	5.1	4.8	240	31	24
0.07	0.08	0.545	0.525	4.4	4.0	240	20	21
0.05	0.06	0.60	0.595	5.3	5.27	720	23	21

With a few exceptions the above runs check each other rather closely as will be brought out in figure 4.

Table VII and table VIII are the same as the two preceding tables except that they are taken from data on the abnormal series.

TABLE VII

MILLILITERS OF ACID AND BASE IN ABNORMAL TISSUE

TIME	3 A		4 A	
	ACID	BASE	ACID	BASE
Minutes	ml.	ml.	ml.	ml.
20	1.054	5.286	1.084	5.476
20	0.886	3.661	0.927	4.07
30	0.763	1.735	0.991	1.85
60	1.303	2.375	1.119	1.314
60	0.716	0.069	0.734	
120	1.028		0.835	
240	1.519		1.00	
240	1.060		0.954	
720	2.611		2.395	
Total 1510	10.940	13.126	10.039	12.710

The above data indicate that there is a deficiency of acid constituents in the abnormal apple peels.

The physical measurements of the abnormal series 3 A and 4 A are presented in table VIII.

TABLE VIII

PHYSICAL MEASUREMENTS OF ABNORMAL APPLE PEELS

AMPERAGE		INITIAL E.M.F.		pH		TIME	TEMPERATURE	
3 A	4 A	3 A	4 A	3 A	4 A	3 A AND 4 A	3 A	4 A
						Minutes	° C.	° C.
1.60	1.65	0.998	0.995	11.91	12.03	20	45	52
1.0	1.05	0.977	0.986	11.73	11.88	20	40	54
0.8	0.7	0.946	0.954	11.20	11.34	30	33	40
0.6	0.51	0.906	0.894	10.52	10.32	60	32	34.5
0.25	0.25	0.709	0.643	7.2	6.08	60	22	28
0.2	0.18	0.626	0.600	5.7	5.35	120	28	19
0.14	0.09	0.585	0.646	5.1	6.13	240	19	20
0.08	0.1	0.549	0.584	4.49	5.08	240	19	25
0.01	0.09	0.555	0.591	4.59	5.2	720	29	22

The data in tables V and VII are presented in graphical form in figure 3. This figure brings out the fact that abnormal apple peels have a significant difference in the dialysable chemical components as compared with normal apple peels. The data in tables VI and VIII are plotted in a similar

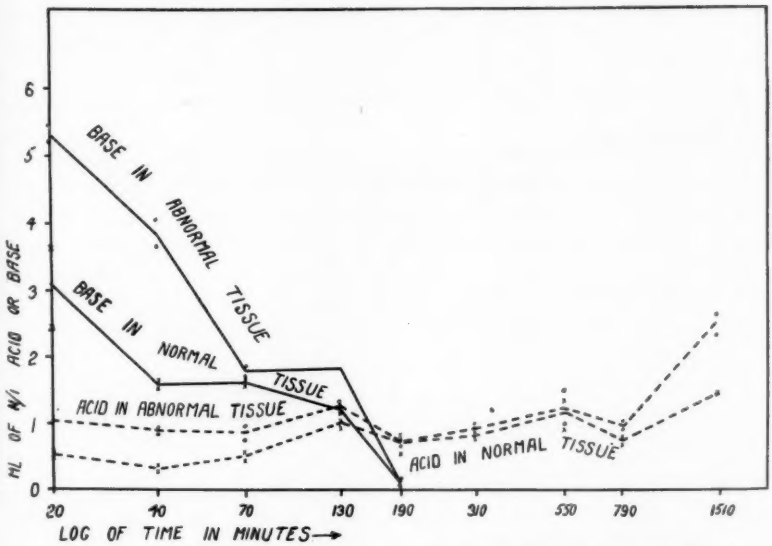

FIG. 3. Milliliters of N/1 acid or base in normal and abnormal apple tissue.

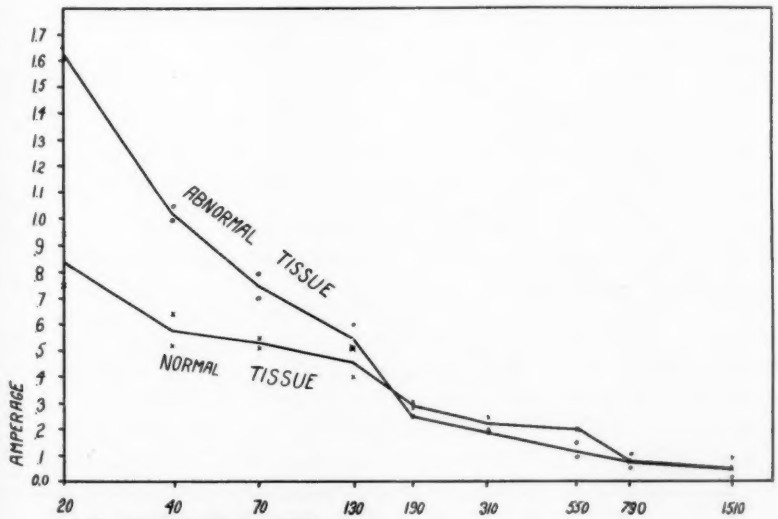

FIG. 4. Amperage plotted against time—normal and abnormal tissue.

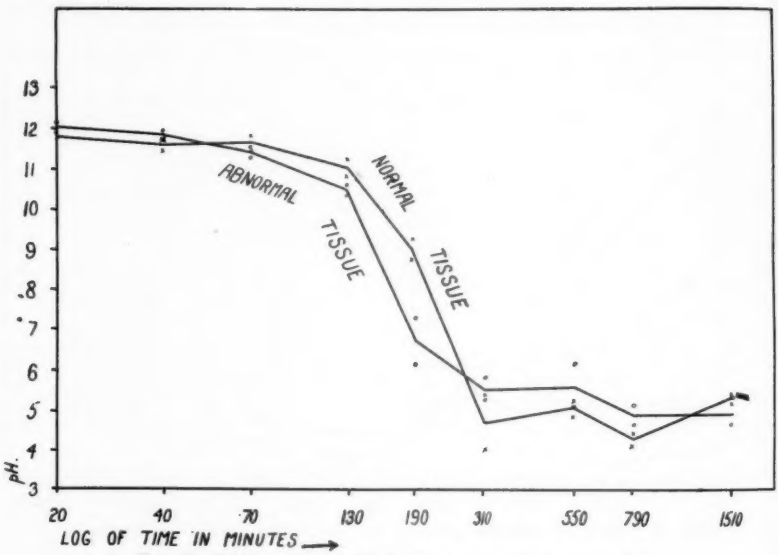

FIG. 5. Initial pH of dialysate at each time interval.

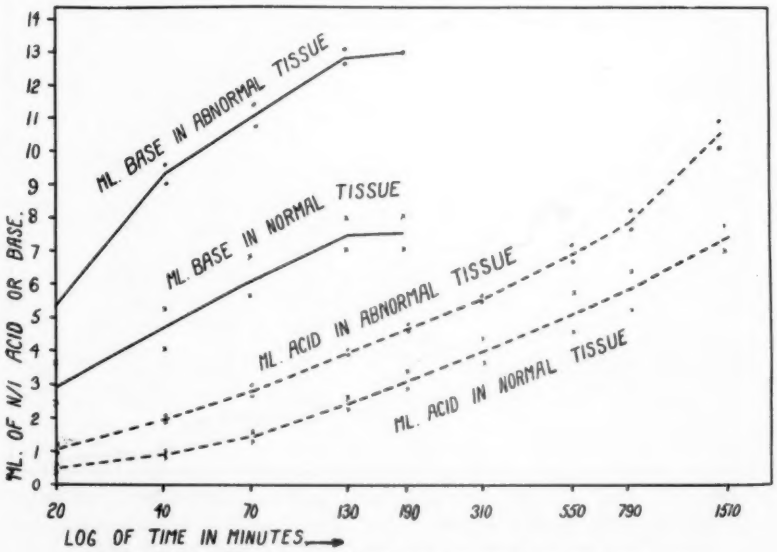

FIG. 6. Total milliliters of acid and base accumulative in elapsed time.

way in figure 4, amperage being plotted arithmetically while time is plotted logarithmetically.

It is interesting to note that the drop in amperage between 130 minutes and 190 minutes corresponds to the removal of the titratable bases as shown in figure 3.

Figure 5 shows the initial pH of the basic solution at the end of each period of time. It should be noted that the normal tissue reaches a pH of 7 after approximately 250 minutes of dialysis, while the abnormal tissue begins much higher and reaches the neutral point much sooner or in about 200 minutes.

Table IX summarizes the data given in tables V and VII. Milliliters of acid and base are indicated accumulatively against elapsed time.

TABLE IX

SUMMARY OF DATA IN TABLES V AND VII

TIME	3 N		4 N		3 A		4 A	
	ACID	BASE	ACID	BASE	ACID	BASE	ACID	BASE
Minutes	ml.	ml.	ml.	ml.	ml.	ml.	ml.	ml.
20	0.688	3.678	0.523	2.440	1.054	5.286	1.084	5.476
40	1.064	5.294	0.826	4.008	1.930	8.947	2.011	9.546
70	1.606	6.885	1.257	5.656	2.693	10.682	3.022	11.396
130	2.652	7.980	2.349	7.016	3.996	13.057	4.121	12.710
190	3.533	8.055	2.900	7.120	4.712	13.126	4.855	
310	4.460		3.698		5.740		5.690	
550	5.846		4.616		7.259		6.690	
790	6.553		5.322		8.319		7.644	
1510	7.930		6.772		10.930		10.039	

The above data are presented graphically in figure 6.

Discussion and summary

In connection with the cold storage investigation being carried on by the Pomology Section of this station, PENTZER (**12**) advanced the theory that Jonathan spot was caused by a loss of acids during storage. The data presented above apparently substantiates PENTZER's theory.

The data reported indicate that electrodialysis offers a convenient means of studying chemical differences in the non-colloidal constituents of normal and abnormal tissue. Quantitative differences which would be masked by large quantities of inert material are accentuated by this method of separating those portions which are soluble and chemically active.

Qualitative analyses on the dialysate indicate that potassium and sodium are removed from the tissue during the first hour. The calcium, magnesium, iron and aluminum are removed after 130 minutes elapsed time.

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GERMINATION AND GROWTH OF SEEDS AS DEPENDENT UPON SELECTIVE IRRADIATION*

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(WITH TWO FIGURES)

The present study of the influence of selective irradiation on germination and growth of the cucumber seed was not undertaken to determine the cytoloplasmic reactions to the component wave-lengths of light, but rather to secure further facts concerning the general response of protoplasm toward wave-lengths of known distribution, with especial emphasis on those responses incited by wave-lengths at or below the shorter end of the visible spectrum. If studies of this sort are to approach the proportions of a science, it is essential that the radiation employed throughout the period of experimental observation be of a known spectral distribution. Results that are attained without due regard for the character of the wave-length employed lend but slight value to the final analysis of the responses incited by any given component. Accordingly, the observations herein reported concern the results obtained in the germination and growth of cucumber seeds under filters of a known transmission, exposed to ultra-violet radiation for given periods of time. The source of the radiation employed was an air-cooled mercury-arc lamp[1] operated at 70 volts at a distance of 50 cm. The lamp was standardized or graded by the method of reaction of the normally unexposed skin of the upper arm to various periods of exposure to the radiation from the lamp at a distance of 45 cm. Three minutes' exposure was required to give a reaction of grade 2 (permanent erythema).

Throughout the experimental period of eight days, germination[2] and growth of the seeds were observed under the following conditions: Cucumber seeds were carefully selected and placed on moist blotters in the bottom of ten medium-sized flower pots. Two of these were covered with ultra-glass (Corning glass, 586 AW), which transmits wave-lengths from approximately 390 mμ to 320 mμ with a maximum of about 370 mμ (fig. 1), and carefully sealed with adhesive tape. For convenience these pots have been

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[1] The lamp used during these observations was made available through the courtesy of the Victor X-Ray Corporation.

[2] The term "germination" is here used to include the earlier growth up to the first appearance of the seedling.

designated Ia and Ib. Likewise, two of the pots were covered with a special vitaglass (made and marketed by F. E. Lamplough, M.A., Birmingham, England), which transmits all of the visible and the lesser wave-lengths down to 270 mμ. These pots have been designated IIa and IIb. Two other pots marked IIIa and IIIb were covered with ordinary window-glass, which

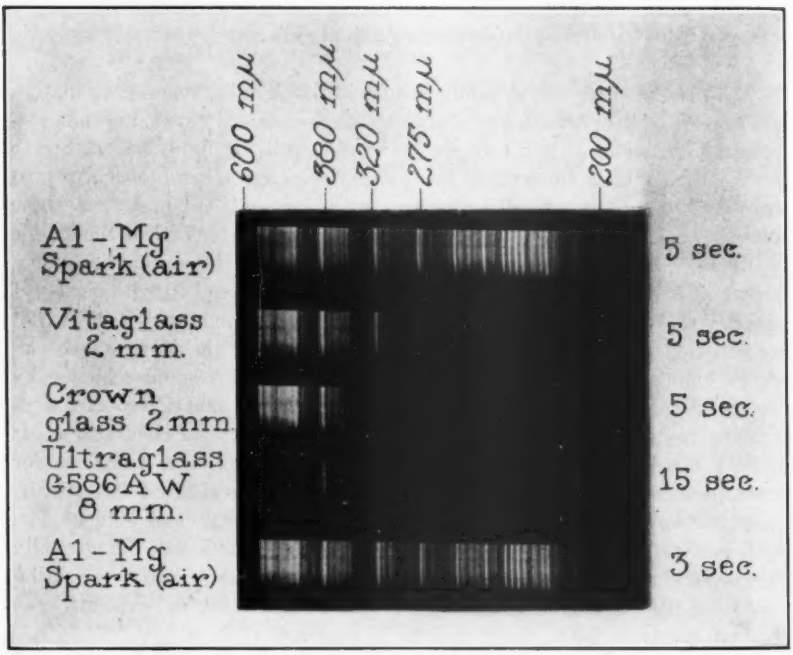

FIG. 1. Spectrograms showing the transmission of visible and ultra-violet radiation by vitaglass (Lamplough, England), ordinary window-glass, and ultra-glass (Corning, 586 AW).

transmits all the visible as well as the lesser wave-lengths down to 320 mμ. Pots IVa and IVb remained uncovered during the periods of irradiation, so that, in addition to the visible regions these seeds were exposed to wave-lengths possibly as low as 190 mμ. In the intervals between the periods of irradiation these pots were covered with ordinary window-glass to prevent evaporation. Pots Va and Vb were not exposed to radiation from the lamp but were covered with ordinary window-glass and served as controls throughout the experiment. Pots Ia, IIa, IIIa, IVa, and Va were kept constantly in a dark cabinet, except for the daily periods of irradiation, while

pots Ib, IIb, IIIb, IVb, and Vb were kept in ordinary daylight under window-glass. Pots Ia and Ib were exposed to the radiation of the quartz mercury-arc lamp for twenty minutes each day, while IIa, IIb, IIIa, IIIb, IVa, and IVb were exposed to the same radiation for five minutes daily. The prolongation of exposure under the ultra-glass was made necessary on account of the absorption of energy due to the greater thickness (see fig. 1). The temperature of the room and of the dark cabinet remained relatively constant during the entire time; that of the cabinet was slightly lower than that of the room. Drops of water were added to the various pots from time to time to maintain a relatively constant degree of humidity.

The experiments here described were repeated five times. Six to ten seeds were used in each container during each set of experiments. In the table and in the illustration (fig. 2) of length of roots under the filters used, we have given sample data which correctly portray the results which were found to occur under the experimental conditions cited in at least eighty per cent. of the cases. We have excluded the few seeds which, for one reason or another, proved to be non-germinating. The experiments were conducted during the months of July and August, 1926, and under conditions of temperature as uniform as possible. The average daily temperature was 75° C. $\pm$ 10°. Hence, in any completed series or group of experiments, all seeds were subjected to identical conditions with the one exception of the variation in the amount and character of the light received.

Observations

Considerable variation in the time of germination of the seeds in the various pots indicated very early that light rather than temperature was the differential factor involved. Observations and measurements of the seedlings were made at the end of the first forty-hour period and at the conclusion of each twenty-four-hour period for eight days. Measurements were all taken at the time of irradiation, so that no additional exposure to daylight was unnecessarily made for this procedure.

At the end of forty hours, with two twenty-minute exposures to the rays of the quartz mercury arc, all seeds in pots Ia and Ib had germinated and the average length of the new growths in each pot was 4.5 mm. Thus far no difference in the respective rates of growth was evident in the pot kept in the dark cabinet and that kept in daylight; but it was strikingly evident that the lesser wave-lengths of these initial exposures, transmitted by the ultra-glass, were sufficient to hasten the time and accelerate the rate of germination.

In pots IIa and IIb, covered with vitaglass which transmits the visible as well as the shorter wave-lengths down to 270 mμ, a difference in the rate

TABLE I

LENGTH IN MILLIMETERS OF SEEDLINGS GROWN UNDER VARYING EXPERIMENTAL CONDITIONS

ELAPSED TIME	I		II		III		IV		V	
	ULTRA-GLASS		VITA-GLASS		ORDINARY GLASS		DIRECT IRRADIATION		CONTROL	
	(A) DARK	(B) LIGHT	(A) DARK	(B) LIGHT	(A) DARK	(B) LIGHT	(A) DARK	(B) LIGHT	(A) DARK	(B) LIGHT
Hours	mm.	mm.	mm.	mm.	mm.	mm.	mm.	mm.	mm.	mm.
40	4	4	3	1	2	0	3	0	0	0
64	14	14	10	6	9	0	7	1	9	0
88	36	24	27	14	23	1	19	5	28	2
112	54	30	42	20	35	2	30	8	52	6
136	65	37	47	25	44	3	31	5	63	8
184	70	40	55	25	60	3	31	5	82	6

Pots I (A) and I (B) were exposed to the radiation of the mercury quartz lamp for twenty minutes each day. Pots II (A) to IV (B) inclusive were irradiated by the same source for five minutes daily.

Ultra-glass (Corning 586 AW) transmits from 390 to 320 mμ with a maximum at 370 mμ. Vitaglass (Lamplough) transmits to 270 mμ; ordinary glass to 320 mμ.

of germination of the seeds was strikingly evident. Those seeds exposed to two five-minute periods of irradiation, and kept in the dark cabinet, had attained an average growth of 3 mm.; while those similarly exposed, but kept in the light, were but 1 mm. long. Here it would seem that the inhibitive effect evidenced in the growth of seeds in pot IIb may be due to the greater wave-lengths to which the seeds were constantly exposed. Although the vitaglass transmits more of the lesser wave-lengths than the ultra-glass, the shortness of the periods of exposure to the lamp, and thus to the visible light as well, was sufficient to counteract the added stimulative effect of the rays below 300 mμ. Then, too, the lethal effect of lesser wave-lengths may have been sufficient to have induced coagulation and impeded normal germination.

A similar difference in the extent of the growth of the seedlings was evident in pots IIIa and IIIb. The average new growth of those seeds, covered by ordinary glass which transmits the visible and lesser wave-lengths down to 320 mμ and exposed to two five-minute periods of irradiation but kept otherwise in the dark, was 2 mm. On the other hand, those seeds similarly exposed, but kept constantly in the daylight under window glass, had not as yet shown signs of germination. This growth of 2 mm. of the seeds in pot IIIa does not equal that of the seeds grown under the ultra-glass, in which case there is an exclusion of all wave-lengths greater than 390 mμ, with a

transmission of a limited region of radiant energy (390 mμ to 320 mμ) in the near-ultraviolet region. In the case of the seeds in pot IIIb, where germination had not occurred, it is probably true that such stimulative action as may have been induced by wave-lengths as low as 320 mμ was in some way inhibited or counteracted by those of the visible spectrum to which the seeds were constantly exposed.

This interpretation is rendered only the more imperative by the evidence gained from seedlings grown in pots IVa and IVb. In these cases, the rays of the mercury-arc lamp were allowed to play directly on the seeds, differing thus from IIIa and IIIb in the absence of any filter. In the intervals between the direct exposures to the lamp, these pots were covered by ordinary glass and kept in their respective light and dark environments. The average growth of the seeds so exposed for two five-minute periods, and kept in the dark, was 3 mm.; while those kept in ordinary daylight showed no evidence of germination. Here, again, it would appear that the stimulative effect of the lesser wave-lengths of the lamp had incited a growth equal to that attained under the vitaglass when kept in the dark. On the other hand, such stimulative action as may have been induced by the periods of exposure was rendered ineffectual by the continued exposure to the greater wave-lengths of daylight. The difference in the growth of the seedlings attained under the vitaglass and the ordinary glass, when kept in daylight, may be explained on the basis that the vitaglass was constantly transmitting certain of the stimulative wave-lengths, while ordinary glass is impervious to those below 320 mμ.

At the conclusion of this first forty-hour period, there was no evidence of germination of any of the seeds either in pots Va or Vb, to which the rays of the mercury-arc lamp had never been applied.

On the basis of these observations certain facts seem evident. The lesser wave-lengths are stimulative and accelerate the rate and time of germination, while the greater wave-lengths are inhibitive and seem to render ineffective the action of the violet and ultra-violet rays. Germination is either induced by the action of the light or by a temperature factor which is accessory to such experiments wherever an air-cooled lamp is employed. Subsequent growth of these seedlings must lead one to conclude that light-rays are in all probability the more inciting factors.

At the conclusion of the ensuing twenty-four-hour period, or sixty-four hours of experimental procedure, with three periods of exposure to the lamp's rays, further changes in the rate of germination and growth were evident. No appreciable difference in the rate of growth of the seedlings has yet occurred in those pots covered with the ultra-glass and kept in the dark and in the light. In pots Ia and Ib the average length of the seedling

was 14 mm., so that wave-lengths up to 400 mμ which are transmitted by the ultra-glass in daylight were not sufficiently inhibitive to counteract the stimulative effect of the ultra-violet rays during the early growth. In pots IIa and IIb, in which the seedlings were exposed to wave-lengths as low as 270 mμ for five minutes each day, the growth of those kept in the dark had exceeded by 60 per cent. that of the seedlings exposed to the greater wave-lengths of daylight. Likewise, those seedlings in pot IIIa, irradiated through window-glass which is impervious to rays shorter than 320 mμ, had attained a growth only slightly less than that attained by the seedlings in IIa in which the wave-lengths as short as 270 mμ were effective. On the other hand, none of the seeds in pot IIIb as yet showed any signs of germination, a further evidence of the counteractive effect of the visible light, which had rendered ineffectual whatever energy had been received by the seeds during the periods of irradiation. Again, in pots IVa and IVb, the same differences in growth just considered for the seedlings in pots IIIa and IIIb obtained. The average growth attained in IVa was 7 mm. as opposed to an average growth of 1 mm. in IVb. The growth in IVb in contrast to the failure of germination of the seeds in IIIb is to be expected, since in the former case the seeds were directly exposed to irradiation, more of the shorter rays being thus made effectual, and a degree of energy secured that was not entirely offset in its effects by the longer rays of the visible to which the seeds were constantly exposed.

The end of this sixty-four-hour period showed most strikingly differences in the rates of germination and growth of the seedlings in pots Va and Vb, the normal controls. With no evidence of germination at the end of forty hours, growth of the seeds in the control pot in the dark had been so rapid as to produce a seedling at this time equal to those grown in pot IIIa, 9 mm. long. All the seeds in pot Vb, the normal control kept in the light, still failed to show signs of germination. The facility with which seeds germinate in the dark is a commonplace observation, and as common is the fact that daylight hinders germination. These observations would seem to point to the conclusion that the energy requisite for germination may be a derivative of the lesser wave-lengths, although no evidence is at present available as to what effect the infra-red may have. In the case of the seeds of pot Va, energy previously stored in the seed by solar irradiation was sufficient to induce germination when unhampered by visible light; but the added energy distributed to the seeds by the exposure to the radiation of the lamp served to increase the normal energy and to accentuate the time and rate of germination.

A greater difference in the germination and growth of the various seedlings was observed at the end of eighty-eight hours (fig. 2). During the last twenty-four hours seedlings grown under the ultra-glass, transmitting wave-

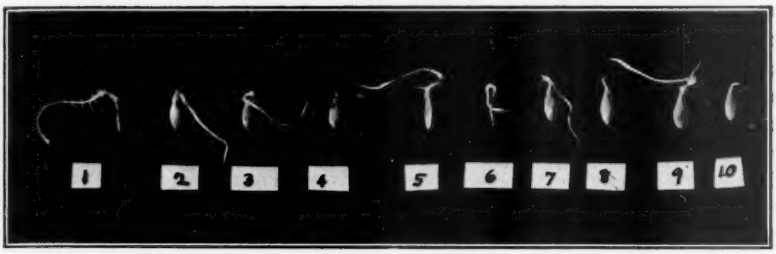

FIG. 2. The extent of growth of the cucumber seed after eighty-eight hours: (1) irradiation of the seed through ultra-glass, seed kept in the dark; (2) irradiation of seed through ultra-glass, seed kept in the light; (3) irradiation of the seed through ordinary glass, seed kept in the dark; (4) irradiation of the seed through ordinary glass, seed kept in the light; (5) irradiation of the seed through vitaglass, seed kept in the dark; (6) irradiation of the seed through vitaglass, seed kept in the light; (7) direct irradiation of the seed, otherwise kept in the dark; (8) direct irradiation of the seed, otherwise kept in the light; (9) normal seedling kept under window-glass in the dark, and (10) normal seedling, kept under window-glass in the light.

lengths of 320 to 390 mμ and kept in the dark, had attained an average length of 36 mm., 50 per cent. more than those kept under ultra-glass in daylight. Likewise seedlings covered by vitaglass which transmits wave-lengths down to 270 mμ had attained twice the growth in the dark that they had in the light. Although, here, the percentage of the lesser wave-lengths transmitted exceeded that of the ultra-glass, yet the inhibiting influence of the visible together with a possible coagulation by the lethal rays would appear to minimize the stimulative effect of the optimal region of lesser wave-length. The seeds within pot IIIb, irradiated for five minutes each day through window-glass, and kept in daylight, had a growth of 1 mm., while those in pot IVb, irradiated directly for the same period of time, and kept equally moist, had attained an average growth of 5 mm. Seedlings in pots IIIa and IVa, kept in the dark except for the five minutes of irradiation, attained a growth of 23 mm. and 19 mm. respectively, only slightly less than the seedlings grown under ultra-glass and vitaglass. Direct irradiation would appear to produce a lethal effect, for the growth in IVa did not quite equal that in IIIa. Then, too, growth of the seedlings in IIIa did not quite equal that of seedlings in IIa, in which the percentage of transmission of the optimal stimulative wave-lengths was greater.

In the normal control pots, Va and Vb, a conspicuous difference was apparent. Those seedlings kept constantly in the dark had attained an average length of 28 mm., while those kept constantly in daylight and under ordinary glass had attained at the end of eighty-eight hours a growth of

but 2 mm. In this case, the seedlings of the dark cabinet exceeded in length all others except those grown under the ultra-glass. If germination and growth are functions of the lesser wave-lengths, as they appear to be, we should expect the growth in pot IIIa, subjected to the ultra-violet irradiation, to exceed that in pot Va. Except for the brief periods of irradiation all conditions were identical. There is, however, a difference of 5 mm. in growth between seedlings grown under identical conditions except for this single variable factor. It is true that seedlings in IIIa received wave-lengths as low as 320 mμ during irradiation, but they were exposed to the visible light for a similar period. And it may be that such exposure was sufficient not only to inhibit the stimulative effects of the shorter rays, but to minimize as well the latent capacity for growth normally stored within a seed. The seeds in Va, given no stimulative exposures, and at the same time kept constantly from the visible rays, were slow in germinating; but the latent stored energy, undisturbed by the antagonistic effects of stimulators and inhibitors, could slowly but gradually come into expression.

Measurements taken at the end of each ensuing twenty-four-hour period for eight days showed that in each experimental condition those seedlings grown in the dark exceeded in length those similarly grown in the light. At the conclusion of the period of observation the greatest difference in the length of the seedling, when the light factor was the only variable, occurred in pots IIIa and IIIb. Here seedlings kept in daylight attained a growth only 5 per cent. of that reached by the seedlings kept in darkness; yet the exposure to the lamp was identical. In the case of those seedlings exposed to direct irradiation by the lamp and kept under ordinary glass, the growth of those kept in the light was only 15 per cent. of that attained by those kept in the dark. Of the controls, those kept in the light were 7 per cent. as long as those kept constantly in the dark. In the cases of the special filters, those seedlings grown under ultra-glass and kept in daylight were 57 per cent. of the length of those in the dark cabinet; while those under vitaglass and in daylight were 45 per cent. as long as those maintained in darkness.

Discussion

The attempt has been made to study the effect of wave-length on the germination and growth of the cucumber seed. Previously it has been shown that the lesser wave-lengths produce a stimulative effect on protoplasm. In studies on the hatching and growth of *Rana*[3] we have shown that the violet and the ultra-violet spectral regions hasten the normal embryologic stages and that certain portions of greater metabolic activity respond more acutely

[3] HIGGINS, GEORGE M., and SHEARD, CHARLES. Effects of ultra-violet radiation on the early larval development of *Rana pipiens*. Jour. Exper. Zool. 46: 333–343. 1926.

to the irradiation. The present experiments on germination have produced results that correspond with more or less exactness to those on the Amphibian ova. In studies on hatchability of the ova the spectral regions employed during the irradiation were not as closely delineated as in these observations on the germinating seeds. In these recent studies filters have been employed to restrict the percentage of wave-length transmission to known regions and thus to establish the influence of selective radiation.

On the basis of the observations and measurements recorded, all other conditions such as temperature and moisture being equal, it is evident that germination and growth are accentuated by radiation in the "near"-ultraviolet region (approximately 400 mμ to 300 mμ). The seeds first to germinate were those exposed to rays transmitted by the ultra-glass filter. Those seeds within the normal control pot, which was kept constantly in the dark and thus subject to the same conditions, did not germinate until twenty-four hours later. Temperature was not a factor, for the ultra-glass employed did not transmit wave-lengths in excess of 400 mμ, and thus we must conclude that the germination was induced by the lesser wave-lengths. In the case of the seeds grown under vitaglass, which transmits wave-lengths as short as 270 mμ, seedlings were never as long at corresponding periods of time as those grown under the ultra-glass. Here, the percentage of transmission of the lesser wave-lengths is greater; but the somewhat lessened growth attained by the seedlings is due perhaps to two factors. Wave-lengths as low as 290 mμ and lower are known to be definitely lethal, and it is probably true that to a certain extent incipient coagulation induced by the exposure to the lethal rays had impeded germination. Likewise, visible light apparently serves to inhibit normal germination. So that in the case of the vitaglass the lessened growth may be due to the inhibiting effect of the visible light or the lethal effect of the lesser wave-lengths.

The radiation from the quartz mercury arc transmitted by window-glass (as short as 320 mμ) seems to inhibit germination of seeds when they are subsequently exposed to solar radiation transmitted through window-glass. In darkness, however, the resident energy within the seed is augmented by such irradiation, so that germination occurs much more rapidly than it does in a similar seed unexposed to the rays of the lamp. Repeated exposures to radiation from the mercury lamp as short as 320 mμ serve to increase the rate of growth of the seedling, so that on the seventh day of experimental observation such seedlings exceeded in length those grown under the vitaglass. Here again, it would seem that the lethal effect of the lesser wave-lengths, which were filtered out by the ordinary glass and transmitted by the vitaglass, was the causal differential factor.

Direct exposures to the rays of the mercury-arc lamp are not ultimately beneficial. Germination, however, is accelerated, and as is to be expected,

a growth is attained, during the first forty hours, equal to that of seedlings under the vitaglass. Subsequent exposure to the direct rays, carrying with it the added lethal effect, is disastrous, for the rate at the end of the second period had decreased and the seedling had attained its maximal growth on the fourth day. Similar conditions obtain for seedlings directly irradiated and kept in the light. Germination occurred sooner than in the normal control group, and the seedling reached its maximal growth on the fourth day. The extent of growth of these seedlings, irradiated directly and kept in the light, is somewhat difficult to understand. A maximal growth of 8 mm. was attained on the fourth day, in contrast to a maximal growth of 3 mm. attained on the fifth day from those seeds irradiated through ordinary glass. The percentage of stimulating wave-lengths falling on the seeds was greater in the former case, and, at the same time, these seeds were irradiated with wave-lengths definitely lethal in character. One must conclude that the lethal effect was more than balanced by the stimulative effect of the optimal wave-lengths.

The normal control seedlings in the dark attained the greatest growth during the period of observation. Germination was somewhat retarded, however, and did not take place until the second period. Subsequent growth nevertheless was exceedingly rapid and during the third period the length exceeded that under the vitaglass, with its daily exposures to the stimulative rays. During the sixth period of observation, the length of the control seedling in the dark reached and surpassed that of those grown under the ultra-glass, thus exceeding in extent all other seedlings grown during the experiment. The control seeds in the light did not germinate until the fourth day and attained a maximal length of 8 mm. during the sixth day, exceeding by 5 mm. the maximal growth of seeds irradiated through ordinary glass and kept in the light. In other words, seedlings appear to thrive better when kept in daylight, if they are not exposed to brief periods of irradiation by the mercury lamp. When irradiated through window-glass, rays as short as 320 mμ are transmitted; but these may be rendered ineffectual by the long waves of the lamp which appear to be even more inhibitory than the visible radiations of daylight as transmitted by ordinary window-glass.

Conclusions

1. Selective irradiation of the cucumber seed modifies the time of its germination and rate of its subsequent growth.

2. Lesser wave-lengths in general appear to stimulate, while the greater wave-lengths inhibit germination.

3. Wave-lengths ranging from about 320 mμ to 390 mμ seem particularly effective in inducing growth.

4. Wave-lengths of 270 mμ to 320 mμ appear to be inhibitory in their action, delay the time and lessen the rate of growth, probably by reason of changes which, carried to their extreme, eventuate in coagulation of the seed albumin.

5. Some of the energy emitted by the lamp and absorbed by the seed may be rendered ineffective by subsequent exposure of the seed to the visible and near infra-red regions of interior daylight.

6. Certain wave-lengths of radiant energy are more potent in germination than temperature. With a constant temperature, germination and growth in the dark greatly exceed those in daylight as transmitted by ordinary window-glass.

7. A certain amount of energy, apparently produced under the action of lesser wave-lengths of sunlight, is normally stored up within seeds. Under proper conditions of light and moisture this energy induces germination.

8. Lesser wave-lengths of light act as stimulative agents which modify the control of endogenous processes and accelerate germination, while subsequent growth and development of the plant is doubtless a function of the visible or infra-red wave-lengths.

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LENGTH OF LIFE OF SEED-PIECE ROOTS OF SUGAR CANE AND PROGRESS OF THE ROOTS IN THE SOIL AT DIFFERENT AGES OF GROWTH

H. ATHERTON LEE AND D. M. WELLER

(WITH FOUR FIGURES)

Introduction

In a previous paper[1] a method of determining quantitatively the distribution in the soil of the roots of sugar cane has been described, and the results of field studies showing such distribution have been presented. Further studies have been made since this previous publication which are recorded in the present paper.

As is perhaps commonly known, sugar cane is propagated on a plantation scale by vegetative cuttings called seed pieces. A seed piece of sugar cane, when it is first planted, puts out roots from the root bands at its nodes at the same time that its eyes or buds germinate. Each eye grows into an aerial shoot but it is some time before such an aerial shoot forms its own nodal roots. During this period such an aerial shoot obtains its nutrients from the vegetative seed piece and through the seed piece from the seed-piece roots. By referring to fig. 1, this can be more readily understood.

The bud of the cane cutting germinates to form an aerial shoot and at the same time the root eyes of the cutting germinate to form roots. The aerial shoot does not form its own roots until it has formed its first cane node and then produces roots from the root band at the node. Until these nodal roots of the stalk are formed, the cane plant functions on nutrients from the seed piece and from the seed-piece roots through the seed piece. Plant A, one month old, shows seed-piece roots formed almost exclusively, while Plant B, three months old, shows stalk roots preponderating over seed-piece roots.

The experiments recorded here show the period in the life of normal young cane plants during which these seed-piece roots function, and the period at which the new cane plant puts out its own nodal roots and functions independently of the seed piece and its roots. The downward progress of the roots into the different levels of soil is also shown from these studies.

[1] LEE, H. ATHERTON. The distribution of the roots of sugar cane in the soil in the Hawaiian Islands. Plant Physiol. **1**: 363–378. 1926.

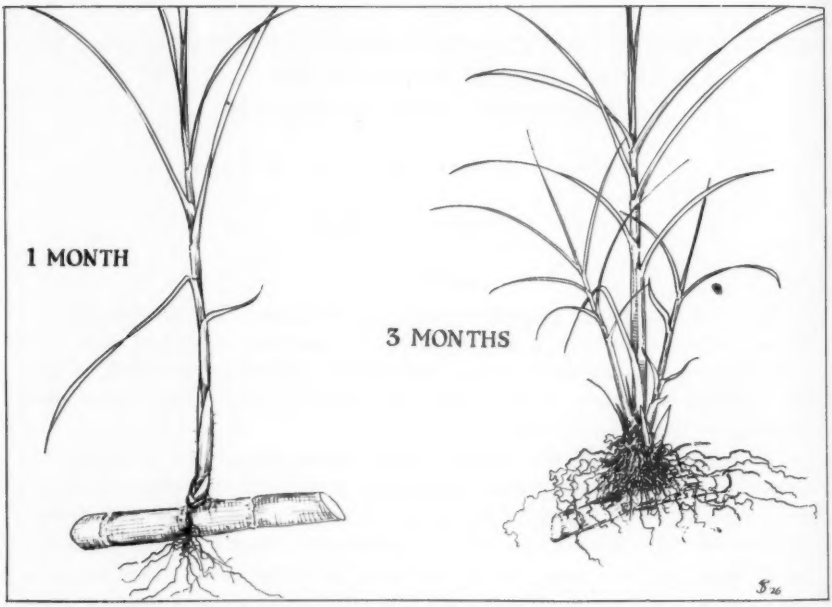

FIG. 1. The method of formation of roots in the early stages of the cane in the experiments.

Methods of study

Fifteen root-study boxes having the removable sides and horizontally placed wire netting, as previously described (*loc. cit.*), were planted each with one seed piece of the variety H 109. These seed pieces were selected for uniformity of length of internodes, diameter, and position on the stalk. Each seed piece consisted of three nodes with their accompanying three buds or eyes; of these three eyes, the two at the extremities were excised, leaving only one bud, or eye, to germinate for each seed piece. At time intervals of one month three of the boxes were taken in consecutive order, according to their position, the sides removed and the soil washed away from the roots of the cane in each box. The roots of the cane were thus left in correct position suspended on the wire netting.

At different levels in depth in the soil, beginning at the bottom and working upwards, the roots were cut off; thus all the roots below the 24-inch level were first cut off and collected. Next the roots between 16 and 24 inches in depth were cut at the 16-inch level, and collected; the roots between 8 and 16 inches in depth were next collected and finally the roots between the soil

TABLE I

WEIGHTS OF ROOTS FROM AERIAL SHOOTS AS COMPARED WITH WEIGHTS OF SEED-PIECE ROOTS OF VARIETY H-109 AT DIFFERENT PERIODS OF GROWTH

AVERAGE WEIGHTS FROM THREE PLANTS OF EACH AGE

LEVELS IN DEPTH	AERIAL-SHOOT ROOTS		SEED-PIECE ROOTS		TOTAL BOTH CLASSES OF ROOTS
CANE 1 MONTH OLD					
	gm.	Per cent.	gm.	Per cent.	gm.
Topmost 8 inches	0.036	4.0	1.094	96.0	1.130
8 to 16 inches	0.00	0.0	0.162	100.0	0.162
16 to 24 inches	0.00	0.0	0.025	100.0	0.025
24 inches downward	0.00	0.0	0.003	100.0	0.003
Totals	0.036		1.284		1.320
Percentages of class in total		2.7		97.3	
CANE 2 MONTHS OLD					
	gm.	Per cent.	gm.	Per cent.	gm.
Topmost 8 inches	9.58	72.5	3.62	27.5	13.20
8 to 16 inches	2.66	88.3	0.35	11.7	3.01
16 to 24 inches	0.94	100.0	0.00	0.0	0.94
24 inches downward	0.33	100.0	0.00	0.0	0.33
Totals	13.51		3.97		17.48
Percentages of class in total		77.3		22.7	
CANE 3 MONTHS OLD					
	gm.	Per cent.	gm.	Per cent.	gm.
Topmost 8 inches	96.75	98.2	1.77	1.8	98.52
8 to 16 inches	29.63	100.0	0.00	0.0	29.63
16 to 24 inches	14.11	100.0	0.00	0.0	14.11
24 inches downward	9.27	100.0	0.00	0.0	9.27
Totals	149.76		1.77		151.53
Percentages of class in total		98.8		1.2	
CANE 4 MONTHS OLD					
	gm.	Per cent.	gm.	Per cent.	gm.
Topmost 8 inches	150.2	99.3	1.0	0.7	151.2
8 to 16 inches	48.6	100.0	0.0	0.0	48.6
16 to 24 inches	27.0	100.0	0.0	0.0	27.0
24 inches downward	24.0	100.0	0.0	0.0	24.0
Totals	249.8		1.0		250.8
Percentages of class in total		99.6		0.4	

TABLE I (*Concluded*)

CANE 5 MONTHS OLD

LEVELS IN DEPTH	AERIAL-SHOOT ROOTS		SEED-PIECE ROOTS		TOTAL BOTH CLASSES OF ROOTS
	gm.	Per cent.	gm.	Per cent.	gm.
Topmost 8 inches	229.0	99.7	0.6	0.3	229.6
8 to 16 inches	61.2	100.0	0.0	0.0	61.2
16 to 24 inches	25.4	100.0	0.0	0.0	25.4
24 inches downward	17.4	100.0	0.0	0.0	17.4
Totals	333.0		0.6		333.6
Percentages of class in total		99.8		0.2	

surface and the 8-inch level. In collecting these roots, those which emanated from the seed piece were carefully separated from the roots originating from the nodes of the aerial shoot or stalk. The separate root collections were then washed more carefully to remove all traces of soil, oven-dried and weighed.

The relation of seed-piece roots to nodal roots of the stalk

Table I shows the weights of the seed-piece roots as compared to the weights of the nodal roots of the cane stalks.

The results recorded in table I show that the cane plant functions entirely by the use of the roots from the seed piece for one month; at the end of one month 97.3 per cent. of the total roots originated from the seed pieces while only 2.7 per cent. of the roots originated from the stalks of the aerial shoots. At the end of two months the situation had changed considerably, only 22.7 per cent. of the roots having arisen from the seed piece as compared with 77.3 per cent. of the roots from the stalks of the aerial shoots. At the end of the third month the situation was completely reversed, with only 1.2 per cent. of the roots emanating from the seed piece and 98.8 from the stalks of the aerial shoots. Thereafter the roots from the seed piece constituted but a negligible proportion of the total roots. The relation of the weights of seed-piece roots to the weights of aerial-shoot roots at different ages of growth is shown graphically in fig. 2.

It is of interest that this change in the proportion of nodal stalk roots to seed-piece roots was not due alone to the increased weight of the nodal stalk roots; after the second month the seed-piece roots did not increase but actually decreased in weight. At the end of the fifth month the seed-piece roots weighed but 0.6 gm. as compared to 333 gm. of nodal stalk roots, amounting to but 0.2 per cent. of the total roots. Since the seed-piece roots

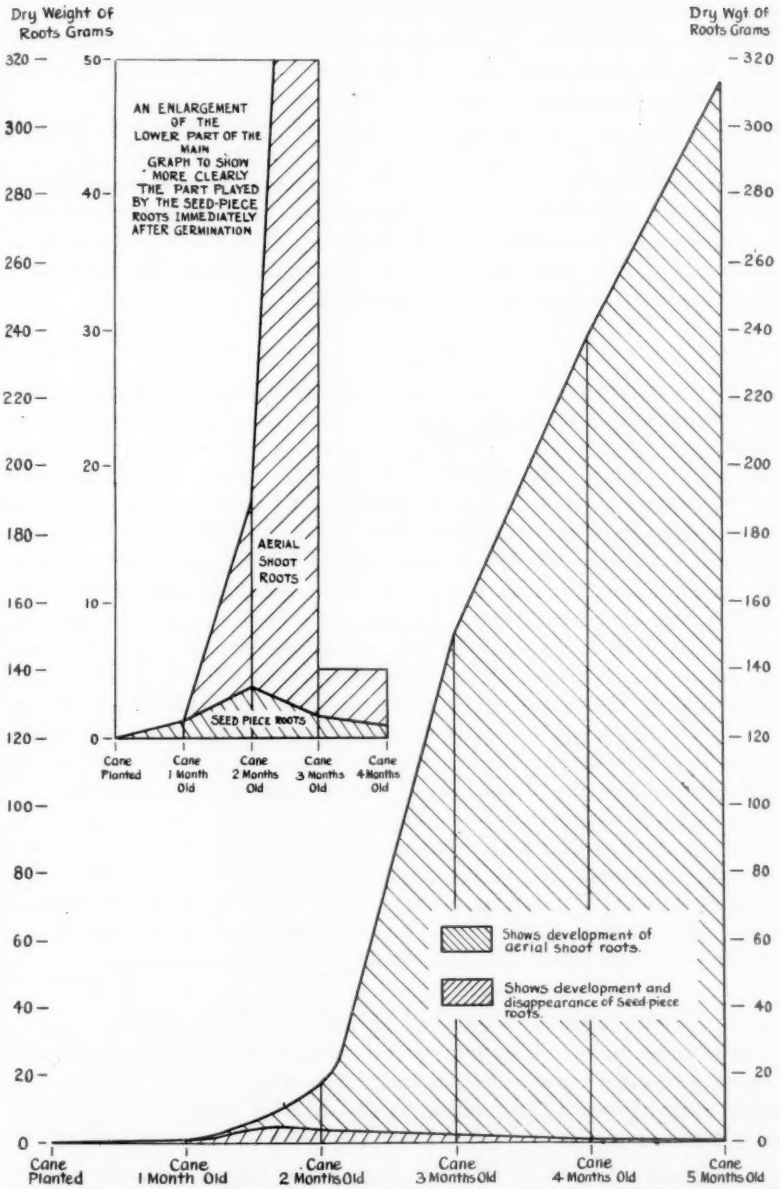
Dry Weight of Roots Grams
Dry Wgt of Roots Grams
AN ENLARGEMENT OF THE LOWER PART OF THE MAIN GRAPH TO SHOW MORE CLEARLY THE PART PLAYED BY THE SEED-PIECE ROOTS IMMEDIATELY AFTER GERMINATION
AERIAL SHOOT ROOTS
SEED PIECE ROOTS
Cane Planted
Cane 1 Month Old
Cane 2 Months Old
Cane 3 Months Old
Cane 4 Months Old
Cane 5 Months Old
Shows development of aerial shoot roots.
Shows development and disappearance of Seed-piece roots.

FIG. 2. The dry weight increase of seed-piece roots as compared to aerial-shoot or stalk roots at different ages in the cane growth.

are not true seminal roots, there is no correlation, nor would any be necessarily expected, between the persistence of the seminal roots of cereal crop plants such as wheat as developed by KRASSOVSKY,[2] and the length of life of roots from these vegetative cuttings of sugar cane.

Therefore under normal conditions *apparently the seed-piece roots alone furnish the nutrients for the aerial shoot for the first month. After the first month and to the end of the second month there is a transition period during which the burden of supplying nutrients shifts from the seed-piece roots to the nodal stalk roots. At the end of the third month and thereafter the burden of supplying nutrients rests almost entirely on the nodal stalk roots since the seed-piece roots have disappeared.*

Discussion

It has been argued from these data that fertilizers should not be applied to the cane until the stalk puts out its own roots, and that fertilizers applied to seed-piece roots will only stimulate roots which will very shortly die and roots will be built up which will be wasted. More careful analysis indicates that there are reasons for early applications of fertilizers which outweigh the foregoing considerations. The aerial shoot cannot form its own roots until it has formed at least one node and the accompanying root band at that node. Thus fertilizers applied early will stimulate the formation of the first node on the aerial shoot and hasten the formation of the first nodal roots. That part of the fertilizer which is not used by the seed-piece roots will remain for utilization by the aerial-shoot roots. That part of the fertilizer used in the formation of the seed-piece roots will not be lost but on the decay of the seed-piece roots will be returned to the soil. One would expect therefore that experiments with nitrogen and potash as well as phosphoric acid, in the furrow, would possibly yield interesting results.

In connection with root-rot studies there is an important conclusion to be drawn, *that one should discriminate between rots of the seed-piece roots after the first month of growth, and rots of the roots from the cane stalk; the decomposition of the former would seem to be a more or less natural feature of the life processes of the cane plant* while, of course, rots in the stele of the roots of the cane stalk would be decidedly abnormal.

The progress in growth of the roots at different ages

In addition to the data showing the comparative weights and proportions of seed-piece roots and stalk roots, data were obtained showing the development of roots of both classes in the different levels in depth in the soil at different ages of the cane. These data are recorded in table II.

[2] KRASSOVSKY, IRENE. Physiological activity of the seminal and nodal roots of crop plants. Soil Science 21: 307–322. 1926.

TABLE II

WEIGHT OF CLEAN OVEN-DRY CANE ROOTS AT THE DIFFERENT LEVELS IN DEPTH OF THE SOIL AT DIFFERENT PERIODS IN THE AGE OF THE CANE

LEVELS IN DEPTH	AGE OF THE CANE									
	1 MONTH		2 MONTHS		3 MONTHS		4 MONTHS		5 MONTHS	
	WEIGHT	PROPORTION	WEIGHT	PROPORTION	WEIGHT	PROPORTION	WEIGHT	PROPORTION	WEIGHT	PROPORTION
	gm.	Per cent.	gm.	Per cent.	gm.	Per cent.	gm.	Per cent.	gm.	Per cent.
Topmost 8 inches	1.130	85.6	13.20	75.5	98.52	65.0	151.2	60.3	229.6	68.8
8 to 16 inches	0.162	12.2	3.01	17.2	29.63	19.5	48.6	19.4	61.2	18.3
16 to 24 inches	0.025	1.9	0.94	5.3	14.11	9.3	27.0	10.7	25.4	7.6
24 inches downward	0.003	0.3	0.33	1.9	9.27	6.1	24.0	9.5	17.4	5.2
Totals	1.320	100.0	17.48	99.9	151.53	99.9	250.8	99.9	333.6	99.9

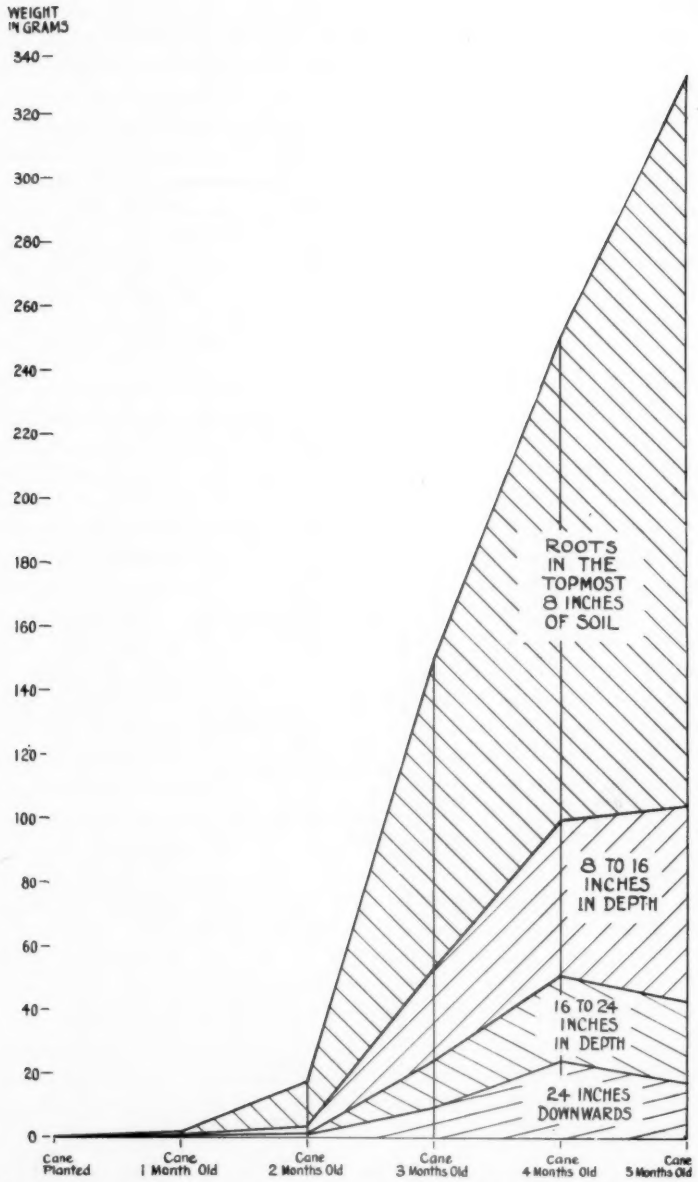

FIG. 3. Dry weight of roots at the different levels in depth in the soil at different periods of growth.

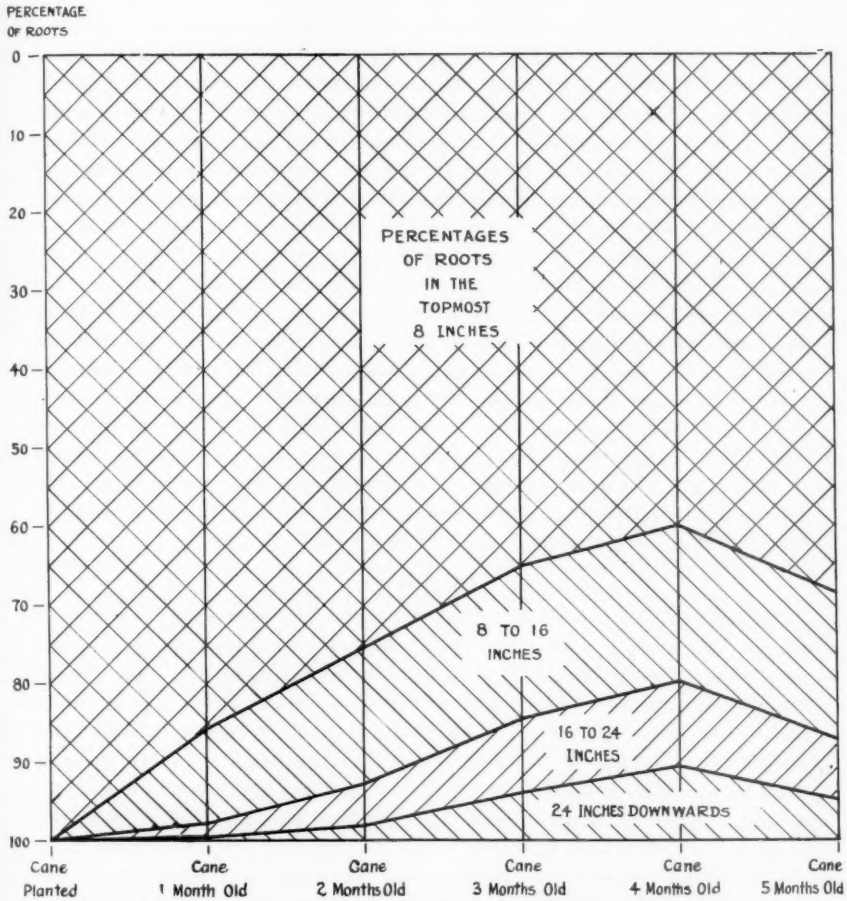

FIG. 4. The percentages of roots of plants of H 109 at different levels in depth in the soils at different ages up to 5 months from planting.

The figures in table II show the combined weights of both seed-piece roots and the roots formed at the nodes of the aerial shoot; the weights, given in grams, are the averages of three plants of each age.

Examining first the figures showing weights, the results indicate what would naturally be expected, that the root weights increased with age, and that the upper levels of the soil were first penetrated and then successively

the lower levels. The weights of the roots in the different levels in depth at the different ages in growth are shown graphically in fig. 3.

The figures concerning percentages of roots are fully as important as the figures for root weights, for in the application of fertilizers and irrigation water, one wishes to place such applications where the largest proportion of the roots exist, and total weights are not as relevant in such questions as are the percentages of roots. If one now refers to the percentages of roots in the different levels in depth in the soil as shown in table II, it can be seen that starting with 85 per cent. of the roots at the end of the first month *the proportions of the roots in the topmost 8 inches of soil gradually decreased until about 60 per cent. of the roots were found in this level; the curve of the decrease then leveled off and it is expected that the plants maintain somewhere between 55 and 70 per cent. of their roots in this stratum until maturity,* at least the results of field root studies (*loc. cit.*) support such a view. At the same time *the percentages of the roots in the lower strata increased to a given proportion and the curve of increase then appeared to level off giving a more or less fixed proportion of the roots through to maturity.* The graph shown in fig. 4 illustrates this approach to fixed proportions of root quantities in the different levels in the soil, after the first few months of growth.

Discussion

It seems to us established from these studies, supported by the field studies previously reported, that water and nutrients, to reach the greatest proportion of roots, should be directed towards the uppermost 16 inches of soil where more than 75 per cent. of the roots usually exist. That tillage and cultivation also need only be shallow seems to us not entirely warranted. As a result of observations during this work on roots we have come to the opinion that, given optimum moisture and nutrients, the outstanding factor for formation of the important secondary roots with their large proportion of feeding surfaces is soil aeration. We do not have quantitative data to support this opinion; our views are based upon field observations only and we present these views as opinion only. If this opinion is correct then deep tillage and organic matter would improve aeration and such improved aeration would increase the feeding surfaces of the roots, thus indirectly increasing cane tonnage. This suggests the desirability of field experiments to test root formation and cane tonnage with increased soil aeration as compared with control conditions.

Summary

1. Based on root weights, a normal sugar cane plant obtains its nutrients during the first month of growth from the seed piece and through

the seed piece from the seed-piece roots. At the end of one month seed-piece roots averaged 97.3 per cent. of the total roots of the plant, and roots from the new aerial shoot or stalk only 2.7 per cent. of the total.

2. At the end of the second month the seed-piece roots constituted only 22.7 per cent. of the total roots, while roots from the new aerial shoots constituted 77.3 per cent. of the total. At the end of the third month but 1.2 per cent. of the total roots were seed-piece roots and 98.8 per cent. of the roots were given off from the aerial shoot or stalk. After this period the seed-piece roots continued to reduce in weight and constituted a negligible proportion of the total roots.

3. A comparison of root weights at different levels in depth in the soil at different periods of growth showed that the quantity of roots in the upper levels of soil increased with the age of the plants; but, while the uppermost roots in the first month of growth constituted fully 85 per cent. of the total roots, yet this percentage gradually decreased during the following month of growth, until only 50 to 75 per cent. of the roots existed in the topmost 8 inches of soil. From that time on, the proportion of roots in the topmost levels of soil became more or less constant.

4. The results presented here suggest further experiments and also give us new points of view in interpreting the results of experimental work dealing with various agricultural practices. There are a number of obvious applications of these results to tillage, cultivation and fertilizer practices.

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FACTORS AFFECTING THE COMPOSITION OF DATES

M. T. Fattah and W. V. Cruess

It is of interest to know whether the composition of the more important commercially grown varieties of dates is affected by the variety and locality where grown and what important changes occur during the ripening process. In some localities, as in Mesopotamia and North Africa, dates mature completely on the trees; in others, because of lack of sufficient heat units during the ripening season, artificial ripening or "processing" is required. Some varieties if allowed to ripen completely on the tree "mummify" and are best if picked slightly unripe and artificially ripened after removal from the tree.

Moisture, total sugars and tannin were taken in our studies as the principal indices of maturity and quality of the dates used.

Effect of locality

Dates are grown in many tropical and sub-tropical countries, among them California and Mesopotamia, the two districts compared in our studies.[1] Growing conditions in these two regions differ considerably. Supposedly mature dates from the two regions were analyzed for moisture, sucrose, reducing sugars and tannin. Some of the data are given in table I.

The Mesopotamian dates were consistently higher in total sugars than were the same varieties grown in California. This possibly means that the dates grown in Mesopotamia ripened fully on the trees, whereas in California, where the heat units during the growing season are said to be somewhat less, the fruit did not reach full maturity or was harvested somewhat sooner than in Mesopotamia in order to reach the market early.

Sugar was determined by the Shaffer-Hartmann[2] volumetric method. Tannin[3] was determined by titration with dilute standard $KMnO_4$, using indigo carmine indicator.

Effect of maturity

Our studies on this phase of the problem were limited to a rather narrow range of maturity, from what might be termed "commercially green" to

[1] Samples of dates were kindly supplied by the Tropical Date Garden and the U. S. Dept. of Agr. Date Garden, of California, and by Fattah and Sons, of Bagdad, Mesopotamia.

[2] Shaffer, P. A., and Hartmann, A. F. The iodometric determination of copper and its use in sugar analysis. Jour. Biol. Chem. 45: 349–390. 1921.

[3] See Official and tentative methods. Assoc. Official Agr. Chemists, 2nd ed., p. 367. 1925.

TABLE I

MOISTURE, SUCROSE, REDUCING SUGARS AND TANNIN IN FLESH OF DATES FROM MESOPOTAMIA AND CALIFORNIA

PERCENTAGE EXPRESSED ON DRY WEIGHT BASIS

SAMPLE	MOISTURE CONTENT	REDUCING SUGARS	SUCROSE	TOTAL SUGARS AS INVERT SUGAR	TANNIN
	Per cent.	Per cent.	Per cent.	Per cent.	Per cent.
Kadrawi, Besia, Mesopotamia	13.9	74.20	0.00	74.20	0.00
Kadrawi, U. S. Date Garden, Calif.	15.4	72.12	1.25	73.43	0.02
Kadrawi, Tropical Date Garden, Calif.	31.6	70.63	2.36	73.11	0.05
Khustawi, Bagdad, Mesopotamia	15.5	77.20	1.52	78.80	0.00
Khustawi, U. S. Date Garden, Calif.	14.6	72.13	0.53	72.66	0.00
Mactum, Bagdad	15.1	80.2	0.00	80.20	0.00
Mactum, U. S. Date Garden, Calif.	17.5	72.15	0.34	72.50	0.00
Zahidi, Bagdad	17.7	83.40	0.76	84.20	0.00
Zahidi, Tropical Date Garden, Calif.	15.6	72.32	1.50	73.89	0.00

"commercially ripe." In all cases the fruit had reached full size. Included also are a few analyses giving a comparison of mummified and soft ripe dates. A few analyses selected from those made are given in table II.

The green dates of all varieties contained considerable quantities of sucrose; with ripening, most of this sugar disappeared (probably by inversion) except in the Deglet Noor variety. The tannin in the green dates was much higher than in the ripened dates.

The mummified sample of Zahidi dates from Mesopotamia is particularly interesting. Although tree ripened, its composition is similar to that of an unripe date. It has 13.11 per cent. of sucrose and about 0.23 per cent. tannin contrasted with 0.76 per cent. sucrose and complete absence of tannin in the soft ripe dates of this variety. Observation shows that dates that mummify on the tree ripen first at the blossom end and ripening proceeds gradually toward the calyx end of the date. In the extremely hot dry air of Bagdad, apparently the calyx end of this variety becomes so dry that ripening is arrested and it remains somewhat immature. Qualitative tests for soluble tannin on the cut surface of longitudinal sections of the mummified Zahidi dates showed considerable tannin in the flesh of the calyx end and none to very little in the blossom end.

TABLE II

COMPARISON OF COMPOSITION OF FLESH OF GREEN, MUMMIFIED AND SOFT RIPE DATES
PERCENTAGE ON DRY WEIGHT BASIS

SAMPLE	MOISTURE	REDUCING SUGARS	SUCROSE	TOTAL SUGARS	TANNIN
	Per cent.	Per cent.	Per cent.	Per cent.	Per cent.
Berhi, Tropical Date Garden, Calif., green	53.8	35.89	16.40	53.15	1.64
Berhi, Tropical Date Garden, Calif., medium ripe	27.8	46.93	15.80	63.56	0.10
Berhi, Tropical Date Garden, Calif., soft ripe	19.7	72.53	0.38	72.93	0.00
Deglet Noor, U. S. Date Garden, Calif., green	42.2	12.60	20.50	41.10	1.90
Deglet Noor, U. S. Date Garden, Calif., medium ripe	29.3	24.70	28.22	54.40	0.21
Deglet Noor, U. S. Date Garden, Calif., soft ripe	18.4	38.20	42.27	82.70	0.20
Deglet Noor, U. S. Date Garden, Calif., soft ripe	20.3	42.00	28.46	71.90	0.20
Hallawi, Tropical Date Garden, Calif., green	52.5	22.20	6.26	28.78	0.50
Hallawi, Tropical Date Garden, Calif., medium ripe	27.0	58.57	1.12	59.74	0.00
Hallawi, Tropical Date Garden, Calif., ripe	21.6	72.62	0.25	72.88	0.00
Kadrawi, Tropical Date Garden, slightly green	48.2	39.80	26.59	67.70	0.60
Kadrawi, Tropical Date Garden, medium ripe	32.20	43.20	24.40	68.51	0.20
Kadrawi, Tropical Date Garden, ripe	31.6	70.63	2.36	73.11	0.05
Zahidi, Tropical Date Garden, Calif., green	53.8	16.72	13.34	30.80	0.70
Zahidi, Tropical Date Garden, Calif., medium ripe	25.9	39.42	12.97	53.07	0.02
Zahidi, Tropical Date Garden, Calif., ripe and partly mummified	17.7	74.25	1.63	75.95	0.00
Zahidi, Bagdad, soft ripe	17.70	83.40	0.76	84.20	0.00
Zahidi, Bagdad, well mummified	14.40	66.80	13.11	76.80	0.23

The above explanation of the presence of appreciable amounts of sucrose in mummified dates does not apply, however, to several samples of Deglet Noor variety that were examined. Although the mummified Deglet Noor dates were rather hard in texture and contained considerably less total sugars than the soft ripe dates, they contained no more tannin than the soft ripe dates. FREEMAN[4] also reported lower total sugar content in mummified than in soft ripe Deglet Noor dates. VINSON has studied the changes occurring in this and in other varieties throughout the growing and ripening period, the comparison being made between a "cane sugar" variety, the Deglet Noor, and various "invert sugar" varieties, principally seedlings.

Effect of variety

The composition of several of the leading varieties of dates is given in tables I and II. They may be grouped in two classes: (1) those high in cane sugar, and (2) those high in invert sugar and low in cane sugar. The Deglet Noor is the principal variety in class 1; most other commercially grown varieties fall into class 2, although when green, VINSON[5] believes all varieties contain appreciable quantities of cane sugar. Our own observations indicate that this is true of the varieties examined. A few additional analyses

TABLE III

ADDITIONAL ANALYSES SHOWING EFFECT OF VARIETY ON COMPOSITION OF DATES
PERCENTAGE OF DRY WEIGHT BASIS

SAMPLE	MOISTURE	INVERT SUGAR	SUCROSE	TOTAL SUGAR	TANNIN
	Per cent.	Per cent.	Per cent.	Per cent.	Per cent.
Ashrasi, Mesopotamia	13.9	66.90	5.98	73.20	0.59
Azrak, Mseopotamia	15.7	69.30	0.28	69.60	0.00
Banawsha, Mesopotamia	15.1	65.60	2.09	67.69	0.00
Degal, Mesopotamia	14.1	59.20	1.62	60.90	0.00
Duck El Badam (mummified), Mesopotamia	10.4	39.00	26.40	66.80	1.80
Kadrawi, Mesopotamia	13.9	74.20	0.00	74.20	0.00
Khalal Matbookh,* Mesopotamia	8.8	22.5	33.63	57.9	0.76

* Cooked immature dates.

[4] FREEMAN, G. F. Ripening dates by incubation. Arizona Exp. Sta. Bull. 66: Part II. 1911.

[5] VINSON, A. E. Arizona Exp. Sta. Bull. 66: Part I. 1911.

showing the effect of variety are given in table III. Of particular interest is the last sample in this table, that of dates that were cooked and dried when slightly immature. Note their low total sugar, high sucrose, and high tannin content.

Changes in composition during incubation at 130° F.

As might be expected immature dates held at 130° F. ripened rapidly with loss of tannin and inversion of sucrose, as shown in table IV.

TABLE IV

CHANGES IN SUGARS AND TANNIN DURING INCUBATION OF IMMATURE BERHI DATES AT 130° F.

TIME	REDUCING SUGARS	SUCROSE	TANNIN
Hours	Per cent.	Per cent.	Per cent.
0	35.89	16.40	1.64
48	43.46	8.75	.03
96	48.64	3.52	.00

After 96 hours the dates were well softened, translucent, amber in appearance and of good eating quality. In this test (repeated with several other varieties with similar results) no attempt was made to remove excess moisture from the dates and they decreased but little in moisture content during the incubation.

TABLE V

CHANGES IN SUGARS, TANNIN AND MOISTURE DURING DEHYDRATION OF BERHI DATES AT 120° F.

PERCENTAGES ON DRY WEIGHT BASIS

TIME	MOISTURE	INVERT SUGAR	CANE SUGAR	TOTAL SUGAR	TANNIN
Hours	Per cent.	Per cent.	Per cent.	Per cent.	Per cent.
0	53.8	35.89	16.40	52.29	1.64
4	43.3	39.80	12.32	52.12	1.10
8	42.5	39.84	12.21	52.05	0.50
12	41.9	40.44	11.51	51.95	0.20
16	39.6	42.22	9.62	51.84	0.11
20	36.9	44.30	7.48	51.78	0.05
24	31.5	47.17	4.53	51.70	0.00
28	29.8	48.96	2.67	51.63	0.00
32	28.5	49.62	0.92	50.54	0.00

Changes during dehydration at 120° F.

Dates are often partially dehydrated before packing. In order to follow the changes in composition occurring during this treatment, Berhi dates (slightly immature) were dried in a blast of air (velocity 500 feet per minute) on screen trays at 120° F. Samples were analyzed at intervals, as given in table V.

Just as during incubation, sucrose decreased greatly and soluble tannin disappeared. After 32 hours the dates were in excellent eating condition. Dehydration was apparently superior to incubation in point of time required, and gave a better product in this test.

Ripening in various gases and vapors

Dates were ripened in various gases and vapors, including CO_2, O_2, CS_2, $CHCl_3$ and C_2H_5OH. Those ripened in CO_2 were normal in all respects and satisfactory in every respect. The changes occurring in sugars and tannin were typical of those in other gases or vapors and are given in table VI.

TABLE VI

CHANGES OCCURRING DURING RIPENING OF BERHI DATES IN CO_2
PERCENTAGE ON DRY WEIGHT BASIS

TIME	REDUCING SUGAR	SUCROSE	TANNIN
Hours	Per cent.	Per cent.	Per cent.
0	35.89	16.40	1.64
48	40.62	11.62	0.50
96	44.76	7.45	0.00

Dates ripened in O_2 were dark brown in color and of poor flavor. Incubation or dehydration at 120–130° accomplished the desired results more satisfactorily than ripening in gases. Dehydration is superior principally in that it permits control of the moisture content of the ripened fruit.

Summary

1. Dates from Mesopotamia used for comparison in this investigation were on the average higher in total sugars on the dry basis and lower in moisture content than the same varieties grown in California, owing perhaps to more favorable ripening conditions (temperature) in Mesopotamia.

2. There was found to be considerable difference in the total sugar content (on dry basis) of different varieties; in some cases this was apparently caused by arrested ripening by mummification (drying on the tree). The Deglet Noor was consistently high in sucrose; most other varieties were low

in this constituent when ripe. All unripe samples of all varieties examined contained considerable sucrose; this decreased greatly during ripening except in the Deglet Noor variety.

3. Soluble tannin was found to decrease markedly during ripening under various experimental conditions, such as during incubation, dehydration, and during storage in various gases and vapors.

4. Dehydration at 120° F. was the most satisfactory means of artificial ripening used in our experiments.

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BRIEF PAPERS

THE EFFECT OF ETHYLENE ON THE RESPIRATION OF BANANAS DURING RIPENING

(WITH ONE FIGURE)

In a paper from this laboratory presented at the Kansas City meeting of the American Association for the Advancement of Science, 1925, it was shown that ethylene doubled or trebled the production of carbon dioxide by celery for a short time after application, and that subsequently the rate fell off to a value below the normal respiratory rate at the same temperature.

Continuing these studies on the physiological influence of unsaturated hydrocarbons in ripening green fruits and vegetables, we have followed the rate of carbon dioxide production by bananas during ripening.

The fruit was placed in sealed glass containers provided with inlet and exit tubes. Suitable wash bottles were inserted to free the incoming air from CO_2 and to keep the air saturated with water vapor. The rate of carbon dioxide production was measured by means of the conductivity cell which was described in the issue of PLANT PHYSIOLOGY for April, 1926. The whole train of apparatus was kept in a constant temperature bath at 25° C. The ADAMS arrangement for the conductivity apparatus was used.

One or two bananas were usually used and the tests lasted from about five to fifteen hours. They were given one or more doses of ethylene carefully measured with a micro-gas burette. The dose was one part of ethylene to one thousand parts of air, since this was the concentration which had been found best suited to ripen bananas. The ethylene was allowed to act for fifteen to twenty minutes and then the aspiration was resumed. The air was aspirated from the container for fifteen to thirty minutes before passing it through the cell, to remove ethylene and the CO_2 which had been liberated during the period of treatment. Conductivity readings were taken every fifteen to thirty minutes thereafter for one or two hours, and if a second dose of ethylene was given to the same specimen, the procedure was repeated. The asterisks on the graph (fig. 1) indicate the points of treatment with ethylene. In all cases the rate of respiration expressed in milligrams of CO_2 per hour was doubled or trebled within a few minutes and then the rate fell off to a value lower than normal.

Bananas from the same bunch and run simultaneously with the treated bananas, although showing some fluctuation in rate, never showed the same

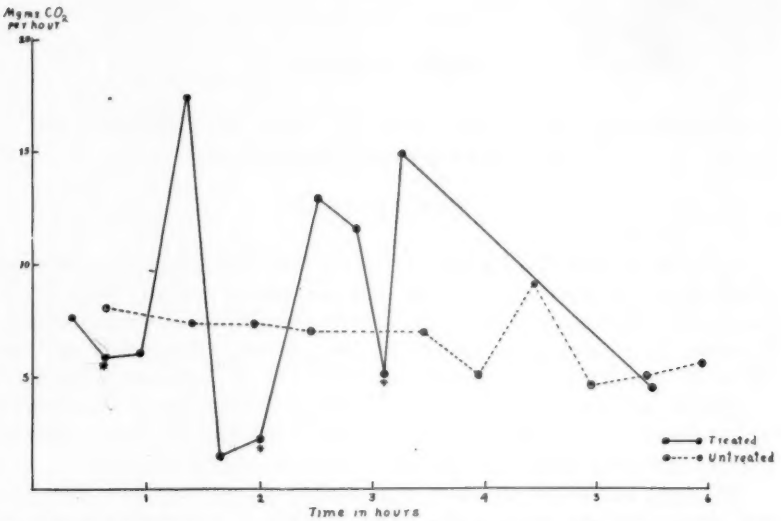


FIG. 1. Respiratory rate in bananas. Ethylene 1–1000 administered at the points indicated by asterisks.

high rates or minima after the passing of the peaks of the curve that the treated bananas did.

Since the trend in all cases tried was the same, it was deemed best to show the graph of one typical treated banana and of one check run simultaneously under exactly the same conditions to illustrate the point rather than attempt to graph several runs on the same paper. This one is typical of many other curves, using Le Gros Michael variety and Cavendish bananas.

The method which was used allowed for the rapid determination of the rate of CO_2 production over a few minutes; consequently it was possible to follow the rapid rise and fall of the respiratory rate better than could be done by the method employed by DENNY[1] on lemons. The high initial rate a few minutes after administration of the ethylene followed by a rapid fall to below normal may be due either to the increase of oxidation or to increase in the permeability of membranes allowing the diffusion of the CO_2 already present in the cells. The rise after the second dose of ethylene seems to indicate an increase in oxidation rate rather than permeability change. Evidently this stimulation wears off within less than an hour.

[1] DENNY, F. E. Effect of ethylene upon respiration of lemons. Bot. Gaz. 77: 322–329. 1924.

The rather rapid removal of ethylene by oxidation, as in the animal body after anaesthesia, offers an explanation for this. Continuous application of the ethylene seems necessary to continued increase in respiration.

Analyses made on treated and untreated bananas show that the treated bananas have one fifth to one fourth more sugar in them than the untreated bananas and that the starch content is proportionately decreased. The activity of the diastatic enzymes as well as the respiratory enzymes is increased by ethylene. Whether this is due to the cell permeability being increased, thereby making it easier for the enzymes and substrate to come together, and to facilitation of the intake of oxygen, or whether ethylene and propylene act as enzyme activators or actually increase the amount of the enzymes, we are now attempting to determine.—L. O. Regeimbal, G. A. Vacha, and R. B. Harvey, *The University of Minnesota.*

AN EFFECTIVE LABORATORY DRIER

(WITH ONE FIGURE)

A rather extended use of the phenol-disulphonic acid method for the determination of soil nitrates led the authors to experiment with various methods of speeding up the necessary step of evaporating aqueous extracts to dryness. The drier finally evolved (fig. 1) has proven fully satisfactory

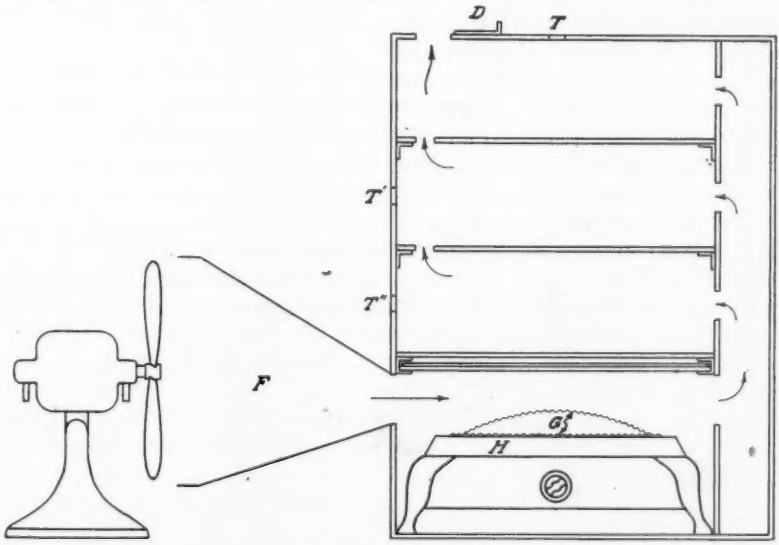


Fig. 1. Vertical section of laboratory drier. Description in text.

not only for that purpose, but also for the rapid drying of green plant tissues for analysis. The principle of operation will be obvious from the illustration. Its important advantages are (1) rapidity, (2) all parts of the drying chamber having very closely the same effectiveness. Details of size and construction may be varied to suit individual needs and preferences, but a brief description will be given of the model we have now in use.

A galvanized iron box, made by a local tinsmith, is lined with asbestos sheeting. The shelving and perforated partition shown at the right are made of asbestos slate (transite) held in place by light angle-irons and stove-bolts. A rapid air draft is provided by a 9-inch desk fan blowing through the funnel *F*, the path of the air being indicated by the arrows. The air passes across *H*, an electric hot-plate, into a narrow chamber at the right, from which it enters the drying chamber through 5 circular openings, 1.5 inches in diameter, opposite the space above each shelf. The second and third shelves do not run the full width of the drying chamber, but leave an air-gap at the left, bridged by light metal pieces (not shown in the figure). A set of air-holes at the top left are fitted with a damper *D* for controlling the draft. The shelf-space is 14 inches square, with 4.5 inches between shelves. With the draft full open and the hot-plate turned to "high" (1,000 watts), the temperature of the drying chamber runs 61° to 63° C. Thermometers are inserted at *T*, *T'* and *T''*, to check the various shelves. It was found necessary to protect the bottom shelf with two extra layers of asbestos sheeting, also to cut down direct radiation by two layers of wire gauze *G* over the hot-plate, to secure the same temperature on all shelves. The narrow chamber at the right is permanently closed in front by the wall of the box. The drying chamber is closed by a glass door, and the hot-plate by a piece of transite containing an opening for the switch. Over both the latter is fitted an outside door of galvanized iron.

Thermostatic control can be added at any time. It has not so far been necessary, as the drier has shown no tendency to vary more than a couple of degrees. In some laboratories it would no doubt be advisable to pass the entering current of air over a moisture absorbing agent. The low humidity of our Alberta atmosphere makes this unnecessary for ordinary purposes.—R. Newton and W. H. Cook, *University of Alberta.*

NOTES

Officers for 1927–1928.—The Secretary of the American Society of Plant Physiologists has announced the results of the recent annual election as follows: President, Professor CHARLES A. SHULL, the University of Chicago; Vice-President, Dr. W. E. TOTTINGHAM, the University of Wisconsin, Madison, Wisconsin. The Secretary-Treasurer, Dr. S. V. EATON, the University of Chicago, was elected last year, and remains in that office.

Midwest Regional Meeting of the American Chemical Society.—The Seventh Midwest Regional Meeting of the A. C. S. was held at Chicago, May 27–28, 1927. The sessions were held in the Kent Chemical Laboratory of the University of Chicago. The meeting was a very valuable one, and was rendered notable by the award of the Willard Gibbs Medal to Dr. JOHN J. ABEL, distinguished biochemist and pharmacologist of Johns Hopkins University, who delivered the Willard Gibbs address on "Chemistry in relation to biology and medicine, with special reference to insulin and other hormones." With the modesty characteristic of really great men he related the history of chemistry in relation to life processes from Paracelsus to modern times, including a brief story of epinephrin and other discoveries of his own, which have brought him international fame. The lecture closed with some of the recent work on isolation of crystalline insulin. The medal was awarded at the Willard Gibbs Banquet, held in Ida Noyes Hall, the presentation address being made by Dr. JULIUS STIEGLITZ, who reviewed in a masterly fashion the scope and magnitude of the work which led to the conferring of this signal honor upon Professor ABEL. The whole meeting was featured by a fine spirit of cordiality, and it will be long remembered by every one who was fortunate enough to be present at the meeting.

The First International Congress of Soil Science.—Students of soil science and related sciences from all over the world met at Washington, D. C., June 13–22, in the first International Congress of Soil Science. Delegates were present from 39 different nations, representing practically all of the important agricultural states of the world.

The meetings were held in the spacious and beautiful halls of the United States Chamber of Commerce Building. The Congress was opened with an address by President COOLIDGE on Monday afternoon, June 13, following which there was a brief response from Dr. J. G. LIPMAN, president of the International Society of Soil Science.

After the first day the morning sessions were devoted to general programs, and the afternoon sessions to the specific work of the various commissions. These meetings of the six commissions were all very valuable, and an account of those of greatest interest to plant physiologists will be presented in the October number of PLANT PHYSIOLOGY.

To relieve the strain of prolonged discussion and presentation of new problems and data, the committee on arrangements provided for a number of field trips and social events, which were much enjoyed. The fourth day, for instance, was taken for a trip by motor bus into Western Maryland and the Shenandoah Valley of Virginia. Other excursions to Baltimore and Mount Vernon added to the attractiveness of the Congress. The trip on the Potomac to Mount Vernon was marred by the fact that the boat was of too deep draught to permit landing, but the river trip in itself was delightful.

Plant physiologists were well represented among those attending the Congress, and many features of the programs were of vital interest to them. The success of the Congress augurs well for future meetings of an international character. The next Congress will convene in Russia, which is only a just recognition of the great part Russian scientists have taken in the development of the science of Pedology.

The Fifth National Colloid Symposium.—The fifth National Colloid Symposium held at the University of Michigan, Ann Arbor, Michigan, June 22-24, 1927, was perhaps the most worth-while meeting since the second symposium in 1924. There was a genuine enthusiasm and considerable spirited discussion, with differences of opinion quite out of the ordinary. There were 250 registered as in attendance, besides a large number of local visitors.

Professor H. R. KRUYT, of the University of Utrecht, was the guest of honor. His paper on "Unity in the theory of Colloids" was greatly appreciated, and the discussion which followed lasted nearly all the morning session and showed the keen interest which his address aroused. Professor KRUYT captivated his audience with his wide knowledge of the subject, his keen wit and ready repartee. He remained at the University of Michigan after the meeting to deliver a series of lectures during the summer session.

The committee on entertainment arranged for auto trips about town and the neighboring countryside; golf at Barton Hills Country Club; a play on Wednesday evening, and the banquet on Thursday evening. A registration fee of $3.00 covered these features, as well as the requirements for admission to the meetings. There were seven papers out of about 25 on the program that were of special interest to plant physiologists. It was re-

gretted by all that DR. LEONOR MICHAELIS was unable to attend the meeting and give his paper on "Investigations on Molecular Sieve Membranes."

The Nashville Meeting.—The meeting of the American Society of Plant Physiologists at Nashville, Tennessee, in December will be the fourth annual meeting of the Society. It should be the largest and most significant meeting so far held by this organization. All members are urged to respond promptly to the requests of the program committee for titles and abstracts of papers to be presented before the sessions at Nashville.

The program committee for this meeting consists of the following members of the Society: Dr. A. L. BAKKE, Iowa State College, chairman; Dr. C. R. BALL, U. S. Department of Agriculture; Dr. W. A. GARDNER, Alabama Polytechnic Institute; Dr. D. R. HOAGLAND, University of California, and Dr. S. V. EATON, University of Chicago. Cooperation with this committee, it is hoped, will be hearty and loyal. The committee invites suggestions which may assist them in planning for a highly profitable meeting.

Stephen Hales.—A short time ago Corpus Christi College, Cambridge University, celebrated the 250th anniversary of the birth (1677) of STEPHEN HALES. This event should focus the attention of plant physiologists on the early history of plant physiology, and the part that STEPHEN HALES played in the development of this branch of botanical science after the Renaissance. His book, *Vegetable Staticks,* published in February, 1727, just a month before the death of Newton, stands as a great landmark in the early eighteenth century; it was the first attempt at a systematic experimental study of an important physiological process, and may justly be considered the chief cornerstone of the historical foundation of vegetable physiology. HALES was 10 years old at the time NEWTON'S *Principia* appeared, and it is quite certain that his association with NEWTON and other great leaders in the Royal Society of London inspired him to become the versatile churchman and scientist that we know him to have been. He was not only a Fellow of the Royal Society, but also "Rector of Farringdon, Hampshire, and Minister of Teddington, Middlesex." The book is "An account of some Statical Experiments on the Sap in Vegetables: Being an essay towards a Natural History of Vegetation. Also, a Specimen of an Attempt to Analyse the Air, by a great Variety of Chymio-Statical Experiments." The experiments were largely devoted to a study of sap pressures (by means of manometers), transpiration, and sap flow.

HALES was not only interested in sap pressure, but studied blood pressure in animals also, and won a permanent place in the history of animal and

human physiology by his studies in that field. He was also an ardent student of ventilation problems, and solved the problem of ventilating ship holds so as to prevent asphyxiation from accumulating fixed air, carbon dioxide. He also invented ventilators for preserving grains from molding, particularly corn.

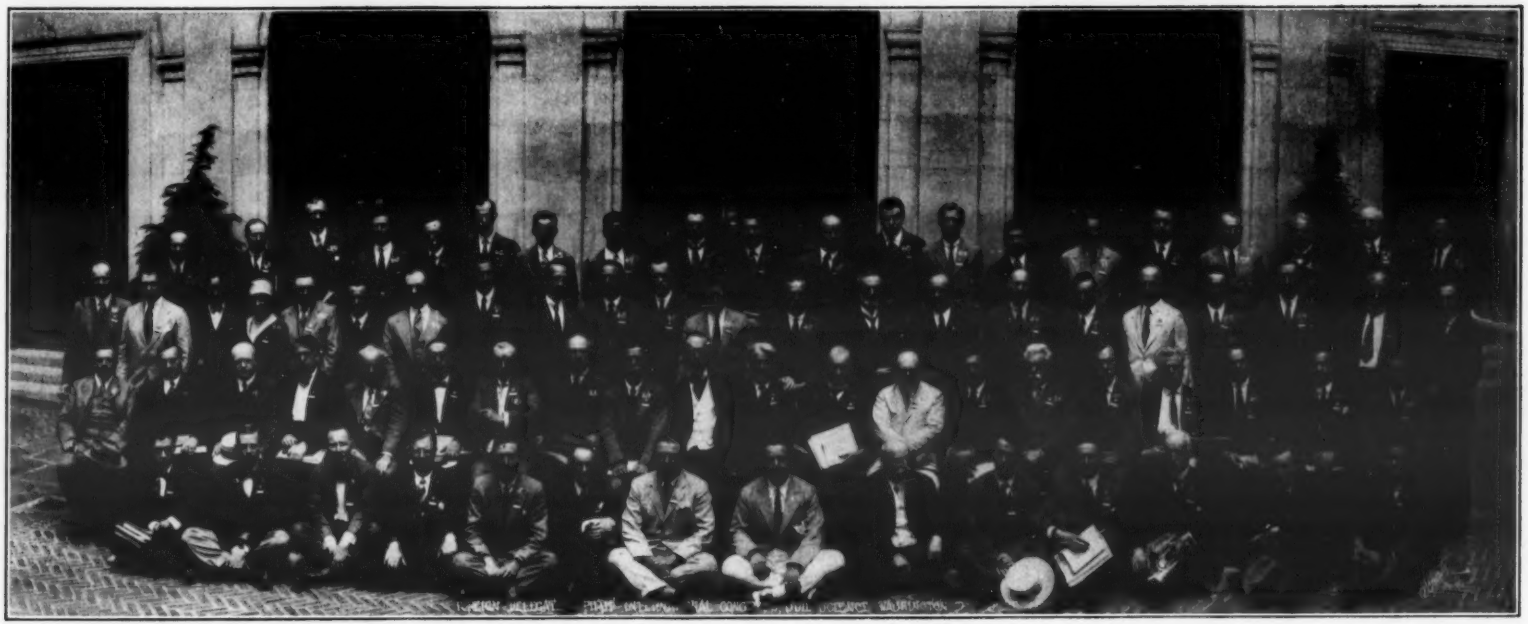
It is an appropriate time to remember HALES, and plant physiologists are proud of the fact that the chief cornerstone in the development of plant physiology, laid just 200 years ago, bears the inscription, "HALES, 1727."

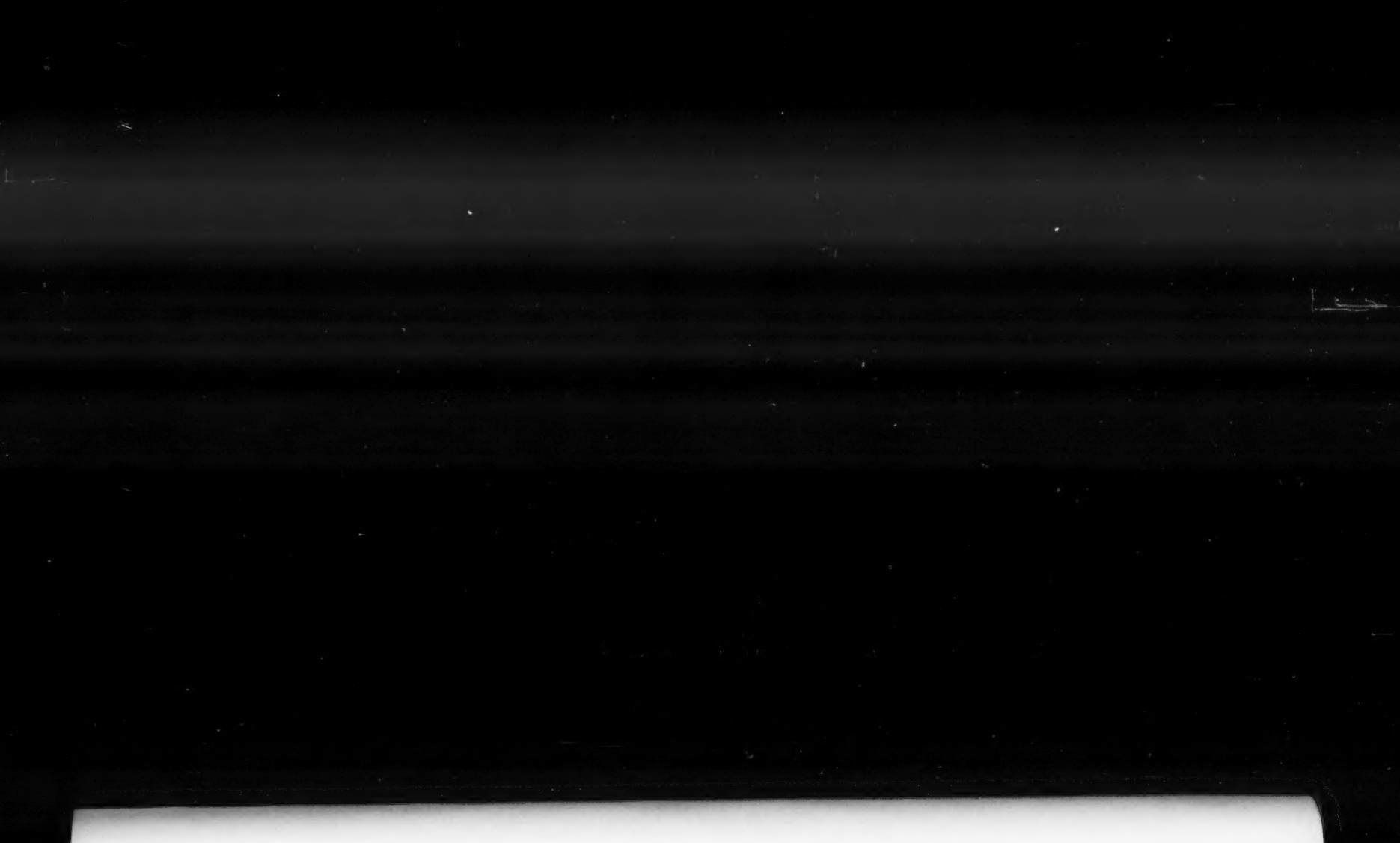
Reports of the Committee on Analytical Methods.—This important committee has published a number of reports during the last two years. Attention is called to the fact that the chairman of the committee, Dr. W. E. TOTTINGHAM, the University of Wisconsin, has made arrangements to supply copies of these reports at a nominal price. The citations to the literature on methods are worth much more than the cost of these separates. Any one desiring to obtain copies of the reports should address the chairman of the committee.

Free Reprints.—Beginning with the year 1928, it is hoped that PLANT PHYSIOLOGY may be able to present a small number of reprints free to each author, provided the author has placed an order for reprints against which the first costs of making the reprints may be charged. The financial support of the journal has been such as to warrant the attempt to give authors some reprints without covers free of charge along with orders. As the support increases, the policy with reference to reprints can be made more liberal.

Plant Autographs and their Revelations.—A popular edition of J. C. BOSE's discoveries in the field of plant responses appears under this title from the press of the Macmillan Co. The characteristic point of view of the author is revealed in the preface where in speaking of the plant, he says: "In order to reveal the intricate mechanism of its life, it is necessary to gain access to the smallest unit of life, the 'life-atom,' and record its throbbing pulsation." Twenty-seven chapters and an appendix tell a marvelous story of sleeping, waking, fatigued, weeping, and nervous plants. If the book makes no more impression upon the lay public than BOSE's previous more serious scientific contributions have made upon scientific men, the book will do little harm. It is unfortunate that such books inevitably sow the seeds of misconceptions of plant life that require decades of effort to eliminate from the minds of uncritical readers. The publisher's price for this popularized edition of BOSE is $2.50.

International Critical Tables.—The second volume of this monumental work has appeared from the McGraw-Hill Book Co. A number of the sections are of interest to biologists. The first section deals with the strength and related properties of woods, from North American, British, Danish, Dutch East Indian, Japanese and Asiatic, Mexican, South American, and West Indian sources. Farther on one finds a section on the durability, chemically, of glasses, which will interest analysts and other users of glass apparatus. Another section deals with animal and vegetable oils, fats, and waxes. Adhesives and gelatins; textile fibers; tanning and vegetable tanning materials; rubber, gutta percha, and balata; artificial plastics, such as nitrocellulose derivatives, and phenol resins and their products; raw materials of the paint and varnish industries; air conditioning; saccharimetry, and X-ray diffraction data are included in this volume, along with much other material of interest mainly to the industries. The largest single section deals with the metals and their alloys, pp. 358–610 being devoted to metallurgy. The volume contains 616 pages, and represents an enormous amount of work on the part of the compilers and editors.



FOREIGN DELEGATES TO THE FIRST INTERNATIONAL CONGRESS OF SOIL SCIENCE

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